

ELEMENTARY MICROTECHNIQUE

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TO
MY WIFE
AND TO
URSULA, JOHN AND NIGEL

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PREFACE TO FIRST EDITION

Much information is stored in many good, but expensive, books to which not all have access. At least I can claim that this book is not expensive, and I trust that, as a consequence, more students will have access to it. From my own experience I have found a need for it.

My object in compiling it has been to fill a gap which to me, both as student and teacher, has always seemed rather big. Students are often supplied with verbal or written instructions for staining and mounting this, that, or the other piece of tissue. Less frequently are they told the reasons underlying the processes, and this is equivalent to working blindfold. Proof that this state of things does exist is evident from the following extract from the *School Science Review* * which appeared during the time when the manuscript of this book was undergoing its first revision :

" . . . The writer is certain that a considerable number of examination candidates fail to make good microscopic preparations for the simple reason that they do not understand why they carry out each part of the process."

I therefore set out to write down, as simply as possible, the whys and wherefores of the processes. During the several years in which I have been collating these notes for publication, my own students have become interested, both in their work and in the progress of the idea, and have encouraged me to carry it out, for they, too, began to see the large gap between scrappy instruction sheets and large and expensive volumes on histological and cytological technique. What they wanted was some concise information in a handy, laboratory-bench form for quick reference. So they began to help—which is better than being merely interested. They tried, and accepted

* T. L. Green, *School Science Review*, XV, 407; March 1934.

or rejected methods of which they read. Some went up to universities and brought back more suggestions. Some tried out new ideas they thought might be useful. And in the meantime I continued collecting and digging out odds and ends.

The result, I believe, is something between the instruction sheet and the large reference book. As I have said, the trouble with big books is that they are so expensive. All the information is not found in one, and most schools cannot afford more than one, and sometimes not that. I therefore hope that this book will prove useful to the people for whom it has been written, namely, Sixth Form and First Year University students. When they have, so to speak, found their feet and learned to walk on the simple methods they will no doubt desire to run on the more advanced methods mentioned in some of the books included in the Appendix.

I have also included a Chapter (VIII), not on any aspect of microtechnique but on the provision of material for histological purposes, and another, (IX), on its preservation.

Some rude person said that Appendices were made not to be read, and if the information was worth while it should be in the book. I leave the reader of the book, and the buyer of the appendix, to decide.

From what I have written it is clear that I owe debts of gratitude to very many people. I mention them not in any order of priority, but rather as their help came to hand. Thanks to numerous lecturers and laboratory stewards—some names unknown and some forgotten—for laboratory notes and instructions stored up over many years; to Messrs. S. V. Baker, C. N. Figgures, B.Sc., J. G. Hawkes (for drawing all the figures, except those mentioned below), A. F. Posnette, D. J. Watterson, B.A., and E. J. Winter—all past or present students—for much help with ideas, suggestions, and information; to my friend Mr. W. J. Clare (of Messrs. R. A. Lister & Co. Ltd., Dursley), for information on the care of razors; to my friend and colleague Mr. F. Craven Broad for much help on the botanical side; and to one, who must remain anonymous, for

reading the MS. and proofs, and for very valuable criticism and encouragement in their production.

There is, I believe, no discredit attached to having read the standard works on a subject, indeed, I would that all for whom this book is intended should make some effort, later on, to read all those books mentioned in the Bibliography. One might describe the state of knowledge of protoplasm and the cell, and of the mode of action of certain fixatives and stains, as one of flux. In the preparation of Chapter I, I have consulted Wilson's *The Cell in Development and Heredity* (Macmillan); Strasburger's *Text-book of Botany* (Macmillan); Piney's *Recent Advances in Microscopy* (Churchill); Conn's *Biological Stains* (U.S. Commission on Standardization of Biological Stains); Carleton's *Histological Technique* (Oxford University Press); Lee's *Microtometist's Vade-Mecum* (Churchill); Chamberlain's *Methods in Plant Histology* (Univ. of Chicago Press); McClung's *Microscopical Technique* (Hoeber); and Baker's *Cytological Technique* (Methuen). The sections on Protoplasm and the Cell, Cell Structure, Fixation and Hardening, Staining, and Clearing in Chapter I, and the Table of the Characteristics and Mode of Action of Fixatives in Chapter II are based (by kind permission of the author and publishers) on the last-mentioned work—a book well worth reading. I would, however, impress the necessity of remembering that, in the biological field, what is thought to be true to-day may be found to be incorrect to-morrow.

I take this opportunity of thanking especially the following authors and publishers for so readily and courteously granting me permission to make use of information in their publications, and should I, by inadvertence, have failed to acknowledge the source of any of my information, I wish, here and now, to tender my humble apologies to those concerned:

Messrs. George Allen and Unwin, publishers of Strasburger's *Handbook of Practical Botany*, for information on Hanging-drop Cultures and the growth of Pollen Tubes; Messrs. Blackie and Sons, publishers of Martin and Johnson's *Practical Microscopy*, for figures incorporated in the table on p. 63; Messrs. J. and A. Churchill, publishers of Lee's *Microtometist's Vade-*

Mecum, for permission to adapt Fig. 17, and information on Embedding Boxes; Messrs. Paul B. Hoeber (publishers), the Oxford University Press (English agents), and Dr. C. E. McClung, editor of *Handbook of Microscopical Technique*, for information therefrom; Mr. Humphrey Milford and the Oxford University Press (publishers), and Dr. H. M. Carleton, author of *Histological Technique*, and Messrs. Hartridge and Haynes, authors of *Histology for Medical Students*, for information supplied; Mr. John Murray (publisher), and Mr. G. H. J. Adlam, editor, for information in Chapters VIII and IX from the *School Science Review*, and for permission to adapt the notes on "Stage Micrometer," "Staining of Chromatin in Fresh Material," and "Mounting Seeds" from the *Science Masters' Book*, Part II; Messrs. Methuen, publishers of Baker's *Cytological Technique*, for information, as indicated above, incorporated in Chapters I and II; and Messrs. W. Watson and Sons, publishers of *The Microscope Record*, for information on the Mounting of Diatoms, and the blocks of Figs. 10, 11, and 15.

No expression of gratitude would be complete without including my publishers, who have shown so much patience and helpfulness in dealing with my suggestions and corrections; and my father and my brother who have both given valuable help and constructive criticism after reading through the MS. in its early stages. Finally, I wish to thank my wife for her unfailing help, encouragement, and patience throughout.

H. ALAN PEACOCK.

BISHOP'S CLEEVE,
October, 1934.

PREFACE TO SECOND EDITION

In this edition the text has been amplified and rearranged in certain parts. For ease of reference the principles and the technique of the various processes have been arranged adjacent to each other. A graded selection of type methods for making microscopic preparations has been added, and it is suggested that a beginner might profitably work through these. The section on the microscope has been rearranged and some information on a simple method for dark-ground illumination has been added.

The uses and formulæ of stains have been grouped together. In view of a certain confusion in stain nomenclature I have used, throughout, the nomenclature adopted by the United States Commission on Standardization of Biological Stains (as given in Conn's *Biological Stains*), and, as an added safeguard when stains are being ordered, I have included the Colour Index Numbers (C.I. No.) of the British Society of Colourists. Only those synonyms in common use in this country (and recognized by the U.S. Commission) have been included, but, as an aid to the accurate description of staining techniques, it would seem preferable to avoid their use altogether.

To Miss E. M. Debenham; Messrs. G. M. Barrett, B.A.; R. W. Bateman, B.Sc.; C. F. Bause; The Cambridge University Press; C. N. Figgures, B.Sc.; Flatters and Garnett Ltd.; T. Gerrard & Co.; A. S. Gillespie, B.Sc.; H. Godwin, M.A., Ph.D.; G. T. Gurr; G. F. Harris; J. G. Hawkes, B.A.; R. Marriott, B.Sc.; C. T. Owen; E. J. Saunders, M.A.; R. Shipway; R. H. Simmons; R. V. Young, B.A.; and to other unnamed readers of the First Edition I owe very grateful thanks for criticisms, suggestions, and information kindly given and freely used. To J. Carpenter I must give my special thanks, not only for help in this direction, but also for so kindly drawing the additional diagrams and for much help with the proofs.

H. A. P.

LECKHAMPTON,
February, 1940.

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HOW TO USE THE BOOK

- *1° Read Chapter I (p. 4) to understand the nature of protoplasm and of the cell. The good craftsman knows the properties of the material in which he is working.
- 2° Read those parts of Chapter II (p. 11) which deal with the principles underlying the processes about to be used. For the sake of convenience, the processes are arranged in the order in which they are used in one of the very common methods with which you will later become familiar. Leave the reading of the parts dealing with technique till you come to the time when you need any particular process.
- 3° Work through the methods given in Chapter IV (p. 71) systematically. Do not pass on to a new method until you have mastered the old and can produce a preparation worthy of you. Have patience and perseverance. Before starting any process, read the technique of carrying it out as explained in the appropriate section of Chapter II. Methods of special use to beginners are shown by a thick vertical line on the left side of the page.
- 4° Before using any stain, familiarize yourself with its components (because these may necessitate a variation in technique) and find out exactly for what the stain is intended and what results you may expect (Chapter VII, p. 220).
- 5° If you are in doubt about the suitability of any of the

* The author first saw this use for the symbol for the degree in Lee's *Microtome's Vade-Mecum* (Churchill) in a table adapted from D. T. Harris's *Practical Histology*. In the present work he has adopted this method for the enumeration of successive stages in the same process. Unless the context implies otherwise, numbers (or letters) within brackets indicate either different processes or alternative methods.

routine methods given in Chapter IV for the particular tissue you are going to use, refer to "Methods for Specific Material," Chapter VI (p. 111). Here you will find variations of the routine methods. If in doubt, use your common sense—you will probably find that a routine method will do quite well, with adjustments to suit your particular case. If you have taken the trouble to use the book in the way suggested you will have sufficient confidence to go forward.

- 6° It is usually cheaper to make up your own reagents if you are using any quantity. Refer to "Formulae and Hints," Chapter VII (p. 220).
- 7° Some sources of material are given in Chapter VIII (p. 291) which also includes some notes on killing certain animal material.
- 8° Sometimes you are confronted with material and wish to preserve it for future use. See Chapter IX (p. 312).
- 9° As time goes on, and you become more interested in the subject, you will find it profitable to refer to the books in the Bibliography (p. 323).
- 10° The following abbreviations are used throughout the book :

alc. . . .	alcoholic solution.
aq. . . .	aqueous solution.
C.I. No. . . .	Colour Index number of the British Society of Colourists.
conc. . . .	concentrated.
dil. . . .	dilute.
esp. . . .	especially.
f(f). . . .	and following page(s).
gr(s). . . .	gram(s).
H.P. . . .	high power.
L.P. . . .	low power.
min(s). . . .	minute(s).
mt. . . .	mount in.
N.A. . . .	numerical aperture.
nuc. . . .	nuclear stain.
perm. . . .	for permanent preparations.

ABBREVIATIONS

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plas. . . .	plasma stain.
p(p). . . .	page(s).
satd. . . .	saturated solution.
satis. . . .	enough to give a saturated solution.
sec(s). . . .	second(s).
soln. . . .	solution.
sp. gr. . . .	specific gravity.
st. . . .	stain with.
temp. . . .	for temporary preparations.
(i), (ii), etc. . . .	alternative methods, or methods for different processes.
(a), (b), etc. . . .	alternative methods, or methods for different processes.
1°, 2°, etc. . . .	successive stages of the same process.
→	result of treatment.

CHAPTER I

PROTOPLASM AND THE CELL

Before trying to understand the processes of microtechnique, it will be well to have some knowledge of the structure and composition of the material, namely protoplasm, in which the work is to be done, and of the principles involved in the processes to which that material is to be subjected.

Protoplasm and the Cell. It may be assumed that the object of making a microscopic preparation is to give the observer as sound a conception as possible of the *living* state of the tissue or cell he is about to examine.

It is only in exceptional circumstances, not likely to be experienced by the beginner, that temporary preparations retain a state in any way approaching the living. For this reason it is necessary to subject the cell or tissue to a series of processes so designed as to enable the tissue to be examined conveniently under the microscope and yet to retain it in as life-like a condition as possible. This seems to be asking a great deal, and actually much confusion has arisen by lack of consideration of the effects on the tissue or cell of the processes to which it has been subjected.

It is not the purpose of this book to make a study of tissues (histology) or of cells (cytology), but before the reactions of tissues and cells to the reagents can be appreciated, the reader must have some idea of the nature of the cell and its contents.

All cells are composed primarily of protoplasm with which may be associated by-products of protoplasm and substances helping to manufacture it.

What is Protoplasm ? There is no completely satisfactory answer to this question because protoplasm is *living*

and in the process of chemical analysis it is killed, and there is no guarantee that what is then found is the same as that which was present in the living protoplasm.

Dead protoplasm is a complex mixture of proteins, fats, carbohydrates, mineral salts and water.

Many theories have been put forward as to the physical nature of *living* protoplasm. Many of these seem to have been based solely on the microscopical appearance of cells after treatment. In other words, the observers were probably looking at artefacts. Modern work with dark ground (oblique) illumination and micro-dissection (i.e. dissection of living cells) points to the conclusion that living protoplasm is a colloidal solution, and belongs to the particular class of colloids called emulsoids. This means that protoplasm consists of two immiscible fluids, each composed of extremely small, shining particles. When an emulsoid is in the hydrosol stage—as is, for example, the protoplasm of leucocytes and germ cells—the more solid of the two components (the dispersed phase) is suspended in the more liquid (the continuous phase) and, due to molecular bombardment, its particles are in a state of constant dancing movement (Brownian motion). If an emulsoid is in the hydrogel stage, the particles of the more fluid component (the dispersed phase) become imprisoned in globules of the more solid component (the continuous phase). This is believed to occur in muscle and nerve cells. Some cells partake of a nature varying from the (hydro)sol to the (hydro)gel condition.

On death, protoplasm becomes a gel, but, unless kept at a temperature of about 0° C., there is then a change to the sol state due to the action of intracellular enzymes in dissolving tissues. The process is called autolysis. This liquefaction is continued under aseptic conditions, and is not to be confused with that caused by putrefying bacteria.

What is the Structure of a Cell? The following summary indicates all that may be found in different cells at different times, and by different methods, and it will be remarked that the present knowledge is scanty and open to qualification.

I. Limiting the cell is :

(A) In most animal cells a living *cell-membrane* said] to contain lipides. (Lipides are substances containing fatty acids combined with some substance usually other than glycerine, e.g. phosphoric acid and/or a nitrogenous base.)

(B) In most plant cells, in addition to the living cell-membrane, an outer, dead *cell-wall* composed of cellulose or, in the fungi, of a horn-like substance resembling chitin, called chitin-cellulose.

II. Constituting the greater portion of the cell-body is the *cytosome* composed of *cytoplasm*. This is protoplasm having the form of a homogeneous liquid composed of aqueous solutions of sugar and inorganic salts, certain sorts of proteins as colloids in the emuloid condition, and about 80% of water. The cytoplasm generally is acidophil(ic) [oxyphil(ic)], i.e. stains with acidic dyes, but may contain particles which are basophil(ic) [basiphil(ic)], i.e. stain with basic dyes.

III. Lying in the cytoplasm are two sorts of *inclusions* :

(A) *Protoplasmic (living) inclusions* which are engaged in metabolism :

- (i) The *central apparatus*, i.e. the *centrosome* (inside which is the smaller *centriole*), which consists of either one or two granules (of unknown chemical composition) said to be the centre of activity of the cell during division. It may or may not be seen during life and is not present even universally in either plant or animal cells.

Concentrated round the centrosome, or sometimes scattered through the cytosome, is the *archoplasm*, an oval area of more dense cytoplasm. It is not always present and is not usually seen during life. From the archoplasm arise the spindle fibres.

- (ii) *The mitochondria (chondriosomes* of plant cells), very small round, or rod-like, or thread-like bodies.* They

* The bodies now known as mitochondria, chromidia, etc., were originally grouped under the collective term "Microsomes." This term is still sometimes used to describe any small granules, not specified, but forming part of the protoplasm as distinct from the metaplasm.

are said to consist of protein and phospholipides and may be seen during life. They can grow, but their functions are not exactly known. It is suggested that they are centres of chemical activity, possibly connected with enzymes.

- (iii) The *Golgi apparatus* (or *bodies*), (" *osmiophilic platelets* " of plant cells), arranged as a reticulum of varying shape, or as a plate, or as curved rodlets, clustered round the archoplasm or scattered through the cytoplasm as a whole. They are larger than the mitochondria and are not seen during life. It is said that they may be composed of a protein combined with a lipide. Though it is known that they can grow, there is doubt as to their functions, but they may be concerned with the secretion of various substances including enzymes.

In connection with the Golgi bodies (which would then be known as *dictyosomes*), there may be an area of cytoplasm called the *idiosome*.

- (iv) The *vacuome*, sometimes called the *neutral red vacuoles* because it includes an aqueous (probably acid) liquid which may be dyed intravitaly with neutral red. It is clustered in or near the Golgi apparatus, or the archoplasm, and may be seen during life. It is not certain whether the vacuome is a protoplasmic inclusion or whether it should more properly be included in the metaplastic (non-living) inclusions (see below). Its function is unknown.
- (v) *Chromatophores* (in plant cells), circular, cigar-shaped, or thread-like. They multiply by direct division :
- (a) *Chloroplasts*, green, flattened, oval granules containing the pigments chlorophyll A, chlorophyll B, carotin, and xanthophyll.
 - (b) *Chromoplasts*, containing the pigments carotin and xanthophyll as minute droplets.
 - (c) *Leucoplasts*, minute bodies of irregular shape. May include albuminous crystals. Concerned with the

conversion of sugar to starch, which latter may appear as granules within the leucoplasts.

- (d) *Pyrenoids*, spherical protoplasmic bodies sometimes associated with chloroplasts, containing an albuminous crystalloid and surrounded by small starch granules.

(B) *Metaplasmic* (or *deutoplasmic*) (*non-living*) *inclusions* which are the products of metabolism :

(i) IN ANIMAL CELLS,

- (a) *Fats* and *oils* in the form of globules. Usually compounds of oleic, palmitic, and stearic acids.
- (b) *Glycogen* as granules or in solution in the cytoplasm.
- (c) *Mucinogen*.
- (d) *Yolk*, in granular or disc-like form. It is a mixture of protein, fats, lecithin, and cholestrin.

(ii) IN PLANT CELLS,

(a) *Liquids*.

- (i) *Cell sap*, occurs in the vacuoles (not the vacuome). May contain acids (citric, malic, and tartaric), albuminous substances, alkaloids, amides, glucosides, glycogen, inulin, mucilage, pigments (anthoclore, anthocyanin, anthophæine), sugars, tannin.

- (ii) *Etherial oils*, *fats*, and *resins*, occurring in vacuoles.

(b) *Solids*.

- (i) *Albuminous substances*, mainly globulins, as *aleurone grains* of round or irregular shape, sometimes containing several crystals.

- (ii) *Calcium carbonate*, as *cystoliths*.

- (iii) *Calcium oxalate*, as single crystals, or as bundles of needle-shaped crystals (*raphides*), or as collections of crystals radiating from a centre (*sphæraphides*).

- (iv) *Inulin*, as *sphærites* (sphere crystals).

(c) *Inclusions in chromatophores*.

- (i) *Albumen crystals*.

- (ii) *Pigments.*
- (iii) *Starch granules*, flat, roundish oval, or spherical.

IV. Separating the cytoplasm from the nucleus (except during cell division) is a homogeneous limiting layer, the *nuclear membrane*, composed of a substance which has been called amphipyrenin.

V. The *nucleus* is a spherical globule composed of *nucleoplasm*, consisting, as far as can be ascertained, of :

(A) *Karyolymph*, a homogeneous aqueous liquid containing proteins in colloidal solution. One of these proteins is

(B) *Chromatin* or *nucleoprotein*, which may be in colloidal solution or in the form of scattered granules, or, during cell division, condensed into *chromosomes*. It forms the greater part of the nucleus.

Nucleoproteins, as a class, are more or less acidic in nature, and hence basophilic. They resist dilute acids but dissolve in dilute alkalis. They are combinations of one or more proteins and nucleic acid (a comparatively strong acid composed, amongst other substances, of a carbohydrate, phosphoric acid, and substances resembling uric acid). Examples of nucleoproteins are :

- (i) *Nuclein*, in which the protein in combination is only mildly basic. Hence nuclein is not definitely a salt and is quite acidic. It occurs in the majority of nuclei which are hence basophilic. The chromosomes are even more acidic and dye even more strongly—hence their name. When fully formed they contain nucleic acid as such.
- (ii) *Nucleohistone*, in which the protein, histone, in combination is more definitely basic and hence nucleohistone is a salt. It occurs in the nuclei of the blood corpuscles of birds, in the sperm of sea urchins, and, with nuclein, in the thymus gland.*
- (iii) *Nucleoprotamine*, in which the protein, protamine, in combination is quite definitely basic and hence nucleoprotamine is a basic salt. It occurs in the heads of the sperms of some fish, including salmon.*

* After Baker, *Cytological Technique*, Methuen.

VI. Within the nucleus may lie one or more sorts of *nucleoli*. These are round or oval bodies of irregular shape and may be seen in the living cell:

(A) *Karyosomes* * (or *chromatic nucleoli*), large aggregations of chromatin and containing nucleic acid. They are basophilic.

(B) *Plasmosomes* † (or *achromatic nucleoli*) composed of a nuclealbumin called plastin or pyrenin which differs from the chromatin and does not contain nucleic acid. They are acidophilic. Their exact function is unknown, but it is probably concerned with the production of yolk-material.

(C) *Nucleolini*, composed of both chromatin and plastin.

VII. Some workers consider that the nucleus throws off bodies—*chromidia* ‡—resembling the cytoplasmic chondriosomes. This view must be accepted with reserve.

* The term karyosome is sometimes used to distinguish the nucleoplasm from the cytoplasm (cytosome).

† The term plasmosome is sometimes used, without distinction, for nucleoli in general.

‡ The bodies now known as mitochondria, chromidia, etc., were originally grouped under the collective term "Microsomes." This term is still sometimes used to describe any small granules, not specified, but forming part of the protoplasm as distinct from the metaplastm.

CHAPTER II

OUTLINES OF PRINCIPLES AND TECHNIQUE

Having obtained some idea of the probable nature of protoplasm and the cell, we proceed to study the processes adopted to enable us "to study the tissue conveniently under the microscope and yet to retain it in as life-like a condition as possible", always bearing in mind that these processes are almost bound to have some effect on the protoplasm, and thus guarding against the danger of forgetting that the artefacts we may see may be only distorted images of the true objects.

In this chapter the processes are arranged in the order in which they occur in the method most likely to be used by the student with some little experience, i.e. Method P-5 (p. 86), for making permanent preparations of counter-stained sections. The statement of the principles underlying a process is followed by notes on its technique. It is suggested that the beginner should first read through the *principles*, leaving his study of the technique of the process until he comes actually to practise it by working through and mastering each of the methods outlined in Chapter IV (p. 71).

The techniques for wax-embedding and for bulk-staining of tissue are given in Chapter V (p. 88).

PREFATORY NOTE TO THE SECTIONS ON TECHNIQUE

Sections and small pieces of tissue are best treated in watch-glasses—about $1\frac{3}{4}$ " in diameter. They should be kept covered by an inverted watch-glass to prevent ingress of dust. Sub-

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sequent location of sections is facilitated if the watch-glasses are set out in a row on a piece of white paper. Mistakes are avoided if the name of the liquid in each vessel is written adjacent to it on the paper, and in the sequence in which each is to be used for the particular method being carried out.

Fixation, washing, dehydration, clearing, and impregnation of tissue in bulk are best done in flat-bottomed capsules or specimen tubes (internal measurements: length, $3\frac{1}{2}$ " ; diameter, $1\frac{1}{8}$ ") fitted with corks. The capsules should be suitably labelled.

Hydration, staining, washing, dehydration, and clearing of sections attached to slides (Chapter V, p. 95) are best done in capsules as above. If the capsules are of the size indicated, they will carry two slides back to back—though the slides are rather liable to cling together. More elaborate apparatus to carry a greater number of slides may be purchased from the usual dealers. A simple device is mentioned on page 283.

Do not omit to label

- (1) the capsules, to indicate the liquid they contain ;
- (2) the inside of the capsules, to indicate the tissues under treatment. This is best done by pencil writing, on a piece of plain white paper slipped inside the capsule.

There is usually no need to throw away the various liquids after once using them. They may mostly (perhaps with the exception of absolute alcohol) be used over and over again, especially if the operations have been carried out in corked capsules. The operator must use his judgment in deciding when the value of any particular liquid is exhausted.

FIXATION AND SUBSEQUENT WASHING

Fixation and Hardening.

This should be carried out as soon as possible after the animal has been killed, or the plant tissue gathered, in order that the tissue shall not shrink by drying, or decompose by putrefaction or autolysis. Methods for killing are, if necessary, given either at the beginning of the special methods for the tissue concerned (Chapter VI, p. 111), or in Chapter VIII (p. 291).

Objects of fixation :

(1) To fix the cell contents in as life-like a condition as possible and so that they may be subjected to further treatment. This may be achieved by precipitation—a physical change (here note that some precipitates are soluble and therefore not fixed), or by coagulation—a chemical change.

In the fixed condition, the cell and its components should be insoluble in the succeeding reagents.

(2) To raise the refractive index of some of the components of the cell or tissue, so that they may be the better distinguished from other components not affected to the same degree.

We can see objects around us because we receive from those objects light of varying intensity and colour. In an unstained or uncoloured tissue we can distinguish different parts only when the light we receive has undergone different treatment by the different parts. This is shortly expressed by saying that the parts visible to us have different refractive indices. Parts of the cell or tissue having the same refractive index will be as invisible to us as a glass rod placed in a mixture of chloral hydrate and glycerine. The glass and the mixture have the same refractive index.

(3) To make the cell resistant to solutions of varying osmotic pressures. Here it may be said that some authorities consider that as soon as the fixative begins to operate, the cell no longer reacts to different osmotic pressures * whether hypertonic (i.e. of osmotic pressure greater than that of the cell contents), or hypotonic (i.e. of osmotic pressure less than that of the cell contents).

(4) To harden the tissue and so enable it to withstand after-treatment—especially by the cutting blade—without distortion. The ordinary fixation processes render most plant and some animal tissues hard enough to be cut into very thin sections, but certain tissues must be submitted to further treatment to make them sufficiently rigid.

(5) To help the tissue to stain better with certain stains. The worker must familiarize himself with the after-effects of

* Tellyesniczky, quoted by Baker, *Cytological Technique*, Methuen.

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fixatives from the point of view of staining and, of course, be careful to use only those stains which give good results after the particular fixative in use, or, conversely, to use only those fixatives which best precede the desired stain(s).

In addition to the foregoing desired effects, there are other undesired effects on the tissue, namely, its shrinkage or swelling to a varying degree according to the fixative used.

While the main object of fixation is to retain the tissue structure unchanged, one may say that, with certain exceptions, its general and immediate cytological effects are :

- (a) to kill the protoplasm (during which the cell or tissue becomes opaque), and alter its physical nature from a hydrogel or hydrosol to a permanent stiff hydrogel, often having, in thin sections, a network appearance.
- (b) to cause a shrinkage of the cell by reason of the precipitant action of the fixative, not because of any high osmotic pressure.
- (c) to precipitate the cytoplasm as a network.
- (d) to remove the vacuome, mitochondria and Golgi bodies.
- (e) to precipitate the chromatin as a network.
- (f) to precipitate the karyolymph as a coagulant which may be stained.

The number and quality of fixatives is many and varied, and the beginner will do well to confine himself to one or two, making sure that he is well acquainted with their use and effects. Some are oxidizing substances, some reducing ; some are acidic, some basic ; some are single substances, some mixtures. When a mixture is used, the object is that certain components shall make up for what one may term the " fixation deficiencies " of the others. The single substance which will, with perfect satisfaction, fix all the cell or tissue components is unknown. Again, some fixatives are better for histological work, some for cytological, and some are equally good for both.

Washing after Fixation.

Object of washing : To remove fixative.

It cannot be too strongly emphasized that thorough wash-

ing in the correct liquid after fixation is essential to good staining. Suitable liquids and times for washing have been included in the table of fixatives (pp. 16-21).

Fixatives.

The more common fixatives are arranged in alphabetical order in the following table, which contains a summary of their main characteristics and mode of use. Where the fixative is a mixture of two or more substances, and no information has been given in certain columns, it may be assumed that their characteristics are by way of being a balance between those of their components. If, otherwise, no information is given, none sufficiently accurate for inclusion is available.

On page 22 are given simple methods which the *beginner should try first*. It will be found that they are quite effective for ordinary purposes. The fixatives suggested for general use in more elementary work are indicated by a thick vertical line to the left of the table.

Certain tissues are best fixed by special methods. Information for these will be found, not in the table, but in Chapter VI, "Methods for Specific Material" (p. 111).

Technique of Fixation.

(1) Before deciding on the fixative, refer to the following summary (pp. 16-21). Only type methods are shown below.

(2) For histological work, cut the tissue into pieces no bigger than 1 cm. \times 1 cm. \times $\frac{1}{2}$ cm. If they can, without distortion, be cut thinner, fixation will be more rapid. Use the fixative and tissue in the proportion, by volume, of 100 to 1 respectively and carry out the process in flat-bottomed specimen tubes (internal measurements: length, $3\frac{1}{2}$ " ; diameter, $1\frac{1}{8}$ ") fitted with corks. Smaller pieces may be treated in watch-glasses.

(3) For successful work, air should be removed from the tissue by carrying out the fixation in a vacuum which may be obtained by means of a filter pump. In cytological work, use the smallest piece of tissue possible.

(4) Remember that, after fixation, thorough washing in a

SUMMARY OF CHARACTERISTICS AND MODE OF USE OF FIXATIVES

<i>Fixative and Main Use.</i>	<i>General Notes.</i>	<i>Penetration, and Time required for Fixation of Small Pieces.</i>	<i>Mode of Washing Fixed Tissue.</i>	<i>Shrinkage or Swelling Effect.</i>	<i>Hardening Effect.</i>	<i>Effect on subsequent Staining.</i>	<i>Mode of Action.</i>
ACETIC ACID. (Animal nuclear cytology.)	Best fixative for nuclei and chromosomes. Cytoplasm and fats not fixed. Action on mitochondria and Golgi bodies uncertain.	Very rapid. About one hour. Must not be allowed to act for long.	Alcohol (50% or 70%).	Swells tissues very much, especially white connective tissue fibres.	None. Leaves tissues soft and incapable of being hardened by alcohol.	Neither helps nor hinders.	Precipitates (in an unlife-like way) nucleoproteins and mucin.
"ACETIC-ALCOHOL."	See Carnoy's Fluid.	—	—	—	—	—	—
ALCOHOL. (ETHYL.) (Animal and plant histology.)	A reducing agent, therefore do not use with tetroxide, or osmium dichromate. Dissolves fats and phospholipides, hence attacks mitochondria and Golgi bodies. Does not fix chromatin. Do not use at very low temperatures. Concentration of alcohol used during and subsequent to the precipitation of glycogen must be at least 50%.	Very rapid. One to three hours.	In alcohol of the same strength as the fixative.	Shrinks tissues very much. Cytoplasm may retract to centre of cell.	Fairly great.	Tissue difficult to stain, but haematoxylin and, for small specimens, alum-carmum, effective. Albumen and globulin tend to stain more easily.	Precipitates both albumen and globulin in an insoluble form, and denatures precipitates nucleic acid and glycogen in a water-soluble form, the latter as a cloud of fine granules, or in amorphous masses.

FIXATION

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BOVIN'S FLUID. (Mammalian histology and animal and plant nuclei cytol. of y.; also for delicate plant and animal material.)	Good fixative for chromosomes. Bad fixative for mammalian kidney. Does not fix mitochondria. Works on embryos dilute with 25% or 50% of its volume of distilled water. A good example of the combination of properties in a fixing mixture. See column 8.	Fairly rapid. Twelve to eighteen hours. (Not more.)	Do not use water. Wash in alcohol (50%) and (70%).	Hardly shrinks cytoplasm. Cf. Acetic Acid, Formaldehyde, and Picric Acid.	Not great.	Tissue easily stainable, especially with Heidenhain's iron-haematoxylin. If using basic aniline dyes, especially safranin O, see that all picric acid is out before staining. Do this by placing sections in lithium carbonate [saturated] in alcohol (70%) (2 min.).	Probably the picric acid checks the action of the formaldehyde and keeps the tissue fairly soft; the formaldehyde checks the action of the picric acid on the cytoplasm, and of the acetic acid on the nucleoproteins; the acetic acid checks the later shrinking effect of the formaldehyde, and fixes the chromosomes.
CARNOY'S FLUID. (Animal cytology, especially chromosomes.)	Fixes cytoplasm and nuclei. Dissolves lipides, Golgi bodies, and sometimes mitochondria. Is not a good fixative for plant tissue.	Rapid. Half an hour. Must not be allowed to act for long.	Alcohol (100%), several changes. Avoid aqueous liquids subsequently.	Shrinkage less than by alcohol, owing to effect of acetic acid.	None.	No deleterious effect on any staining method.	Cytoplasm fixed and glycogen precipitated by alcohol. Nucleoprotein fixed by acetic acid.
CHROM-ACETIC. (Plant histology.)	Does not precipitate karyolymph, nor some cytoplasmic elements. Useful for annelids.	Slow but good. Twenty-four hours, but longer time has no ill effects. Pieces of tissue must be very small, if possible not thicker than $\frac{1}{16}$ in.	Running water twenty-four hours, then distilled water, then twelve hours, then transfer to alcohol (50% or 70%, or 90%).	Little change. Swelling by acetic acid counteracted by shrinkage due to chromic acid.		No deleterious effects.	
				Cf.	Acetic Acid and Chromic Acid.		

* See "Formol-Saline—General Notes," and footnote † on p. 19.

<i>Fixative and Main Uses.</i>	<i>General Notes.</i>	<i>Penetration, and Time required for Fixation of Small Pieces.</i>	<i>Mode of Washing Fixed Tissue.</i>	<i>Shrinkage or Swelling Effect.</i>	<i>Hardening Effect.</i>	<i>Effect on subsequent Staining.</i>	<i>Mode of Action.</i>
CHROMIC ACID. (Animal cytology, and plant histology, especially algae).*	Weakly acid. An oxidizer, therefore do not use with alcohol or formaldehyde. Does not fix fats, but causes globules to coalesce. Does not fix lipides. Destroys mitochondria. Light may cause outer pieces of fixed tissue to dissolve. Fixes outward form and internal structure.	Fairly slow. Twelve to twenty - four hours. Pieces of tissue must be very small, if possible not thicker than $\frac{1}{8}$ in.	Wash thoroughly for twelve hours in running water. Transfer plant material to alcohol (50% or 70%, or 90%).	Shrinkage of nucleus medium, but of cytoplasm considerable.	Medium.	Helps staining when basic dyes are used.	Precipitates all proteins as insoluble compounds, and chromatin as a reticulum.
"CORROSIVE-ACETIC." (Histology of very small animals).*	A very useful fixative. Differentiation helped.	Very rapid. About one hour.	As for mercuric chloride.	Shrinkage by mercuric chloride is counterbalanced by the swelling due to acetic acid.		No deleterious effect. Carmine stains are especially good.	
"CORROSIVE-FORMALDEHYDE." (Animal histology, and some cytology).*	Preserves with minimum distortion. Useful for red and white blood corpuscles.	Fairly rapid. Twelve hours.	As for mercuric chloride.	Cf.	Acetic Acid and Mercuric Chloride.		
				Cf.	Formaldehyde and Mercuric Chloride.		

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"CORROSIVE"
SUBIMATE.

	See Mercuric Chloride.	Slow. One to two days.	Do not use water. Wash in alcohol (70% or 90%).	Little shrinkage at the time, but great shrinkage afterwards in alcohol (90%, or 100%). <i>Arazi</i> shrinkage with mammalian testis.	Very great (probably by acting on lipides of cell membrane). Does not harden but prevents its subsequent hardening by alcohol. (Baker, <i>Cytological Technique</i> .)	Helps staining by making eosinophilic. Makes staining by acid dyes (e.g. eosin Y) difficult.	Up to 4% does not precipitate. Greater strength precipitates very little more. It combines with proteins.
FORMALDEHYDE. (Animal histology).*	A reducer, therefore do not use with chromic acid, or osmium tetroxide, or potassium dichromate. Does not make albumen insoluble. No effect on fats. Makes outline of nucleus less clear. Mitochondria appear as rows of spheres. Do not use for mammalian testis. (Great shrinkage—reason unknown.)						
FORMALIN-ALCOHOL. (Plant histology.)	A good general fixative. For rapid fixation of small pieces, or sections, of fresh tissue, algae. Material may be left in fixative till required for use.	Rapid. Fifteen minutes for small pieces. Longer for larger pieces.	Alcohol (70%). Small pieces, two minutes.		Cf. Alcohol and Formaldehyde.		
FORMAL-SALINE. See Formaldehyde.	Opinions differ as to the advisability of diluting the formaldehyde with saline instead of water, but Carleton (<i>Histological Technique</i>) considers it gives better results.†	Slow. Twenty-four hours.			Cf. Formaldehyde.		
HEILY'S FLUID.	See Zenker-Formol.						

* See "Formol-Saline—General Notes," and footnote below.

† Carleton's work has since been confirmed by Young (*Nature*, 135, 824; May 18, 1935), who recommends that all fixatives for marine animals should be made up in isotonic saline.

<i>Fixative and Main Uses.</i>	<i>General Notes.</i>	<i>Penetration, and Time required for Fixation of Small Pieces.</i>	<i>Mode of Washing Fixed Tissue.</i>	<i>Shrinkage or Swelling Effect.</i>	<i>Hardening Effect.</i>	<i>Effect on subsequent Staining.</i>	<i>Mode of Action.</i>
MERCURIC-CHLORIDE. (Animal histology and plant cytology.)*	May be mixed with the other fixatives. No effect on lipides and mitochondria. Fat globules coalesce. Nucleus fixed in an unlife-like manner.	Rapid. One to two hours.	Wash in alcohol (70%) made the colour of sherry by the addition of a saturated solution of iodine in alcohol (70%). As colour disappears replace by fresh iodine solution. Remove iodine colour with "hypo."	Shrinkage of tissues fairly considerable and that of cytoplasm great.	Moderate.	Staining especially with carmine, easy, and with safranin O and haematoxylin quite good.	Precipitates proteins. Precipitates are soluble in saturated sodium chloride and potassium iodide. Fixes by denaturing (see mode of action of alcohol) the proteins. Fixes nucleus in an unlife-like manner.
"OSMIC ACID."	See Osmium Tetroxide.	—	—	—	—	—	—
OSMIUM TETROXIDE. (Animal cytology, especially microscopic animals; plant histology.)*	An oxidizer. Do not mix with alcohol or formaldehyde. Fixes cytoplasm in a life-like way, but nuclei badly. Does not precipitate proteins. Fixes fats, lipides, and mitochondria. If acidified with 15% of glacial acetic acid it fixes cytoplasm well but nucleoproteins in an unlife-like way.	Rapid, but uneven. Action on proteins slow. One to two days. If vapour is used for small animals, thirty seconds.	Running water, twelve hours. Slight washing with water after use of vapour.	Swelling slight but may shrink after subsequent treatment.	Slight.	Hinders staining, but methyl green in haematoxylin useful.	The unsaturated fatty bodies are oxidized, reducing the peroxide to oxide which forms black compounds with the fatty substances.
PICRIC ACID.* (Animal cytology; plant histology, especially algae.)	Fairly strong acid. May be mixed with any other fixatives mentioned. No effect on lipides. Fat globules coalesce. Mitochondria appear as rows of spheres.	Fairly rapid. Three to four hours. Very small objects, two minutes; large pieces, twenty - four hours.	Wash with alcohol (50% or 70%, or 90%). See "Bouin's fluid" for treatment of picric acid fixed tissue.	Great shrinkage.	Very little.	Staining easy, especially with picro-carmin.	Precipitates all proteins by formation of protein plicates, all insoluble in water.

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"PICRIC-ALCOHOL." (Plant histology, especially algae.)	Fairly rapid. Three to four hours.	As picric acid.	←	→	No deleterious effect. Potassium carmine especially good.
POTASSIUM DICHROMATE (Animal cytology.)*	Best not used by itself because chromatin is dissolved. Cytoplasm fixed in a lifelike fashion. An oxidizer, therefore do not use with alcohol or formaldehyde. If acidified it fixes like chromic acid. Fats not affected. Mitochondria fixed but tend to thicken.	Fairly slow, especially action on proteins. Therefore fix for several days.	Running water, twelve hours. Avoid alcohol till after staining, especially with carmine.	Shrinkage little, but may increase during subsequent operations.	Slow, but good effect.
WATER (HOT). (Animal histology)	A rapid method for very small pieces of tissue when the work is urgent and especially good results are not required. Temperature should be about 90° C.	One minute.	Transfer to alcohol (70%).		Probably fixes by combination. Does not precipitate proteins when used by itself but renders them insoluble.
ZENKER-FORMAL. (Vertebrate histology.)*	Although this fluid contains a mixture of an oxidizing agent (potassium dichromate) and a reducing agent (formaldehyde), it is a good fixative for the purpose indicated. Make up fresh before use. Not good for plant tissues.	Moderately slow. Twelve to twenty-four hours.	Running water sixteen hours, then as for mercuric chloride (q.v.).	Rather liable to distort certain tissues. Cf. Formaldehyde, Mercuric Chloride and Potassium Dichromate.	Albumen coagulated.

* See "Formol-Saline—General Notes," and footnote thereto, p. 19.

22 OUTLINES OF PRINCIPLES AND TECHNIQUE

suitable medium is absolutely essential if subsequent processes are not to be ruined. This is especially true when mercuric chloride and acid fixatives have been used. Even after apparent thorough washing, acid has been known to come out of the tissue when in storage, and, as a result, successful staining has been impossible. As a precaution, always re-wash stored material prior to working with it.

A. Animal Tissue.*

(a) *For very rapid fixation of small pieces of fresh tissue.*

1° Drop into hot water at 90° C. (p. 21). (1 min.).

2° Transfer to alcohol (70%). (1 min.).

(b) *For general use.*

1° Cut the tissue into pieces not larger than 1 cm. × 1 cm. × $\frac{1}{2}$ cm.

2° Transfer to formol-saline (strong) (pp. 19, 248). (24 hours).

3° Transfer to alcohol (50%), or (70%), or (90%).
Avoid water.

(c) *For protozoa, Paramecium, Vorticella, spermatozoa, cœlentera, Hydra, polyzoa, and aquatic organisms, see Chapter VI.*

(d) *See also "Animal Tissue," Chapter VI (p. 116).*

B. Plant Tissue.

(a) *For rapid fixation of small pieces or sections of fresh tissue; algæ.*

1° Formalin-alcohol (pp. 19, 247). (15 mins.).

2° Alcohol (70%). (2 mins.).

(b) *For general use.*

1° Cut the tissue into pieces not larger than 1 cm. × 1 cm. × $\frac{1}{2}$ cm.

2° Chrom-acetic (p. 17). (24 hours).

3° Wash in running water. (12 hours).

(c) *See also "Plant Tissue," Chapter VI (p. 193).*

* See "Formol-Saline—General Notes," and footnote thereto, p. 19.

SECTIONING

Most pieces of tissue are too thick to allow sufficient light to pass through them for microscopic examination. Exceptions are very thin small whole objects, pieces of very thin tissue, and liquid or semi-liquid tissues which may be thinly smeared on a glass slide or cover-slip.

Generally it is found necessary to cut exceedingly thin sections of the tissue. With practice, it is possible to cut quite thin sections with a razor, free-hand, provided the tissue can somehow be held firmly. More accurate work and thinner sections can be obtained by placing a block of embedded tissue in a microtome. This is a device which, at once, holds the block rigid, delivers a definite quantity of block for sectioning by the razor blade, and incorporates the cutting blade. The instrument may be a hand-microtome, when the delivery and sectioning devices are manipulated by hand operation, or a mechanical microtome, when all the operations are carried out automatically at one operation of the machine. (See Chapter V, p. 92.)

Free-hand sectioning is quite satisfactory for fairly stiff plant tissue if the material has been fixed and hardened before-hand. It is rarely completely satisfactory for most animal tissues owing to their general lack of rigidity. It is very moderately satisfactory if the tissue has been well hardened during fixation.

Technique of Free-Hand Sectioning.

The razor must be of shaving sharpness. For ordinary work, sections should be from 8μ to 12μ thick ($\mu = \frac{1}{1000}$ m.m.). To strop a razor see "Razor, to sharpen" (p. 275).

- 1° Hold the tissue firmly between two pieces of carrot (p. 234) or elder pith (p. 244) which has been stored in alcohol (70%).
- 2° Place on the razor some of the liquid in which the tissue has been standing and cut through carrot and tissue, keeping the razor horizontal and drawing it towards the body with a long, oblique, sliding

movement. Do not stop at one section ; cut several before proceeding to 3°.

When cutting transverse sections of plant stems and roots do not, at first, attempt to cut complete sections. Be content with the thinnest representative sectors you can cut at right-angles to the main axis of the tissue.

- 3° By means of a camel-hair brush quickly transfer sections to more of the liquid in which the tissue has been standing.
- 4° Select sufficient of the thinnest sections and transfer them
 - (a) for a temporary preparation (i.e. one shortly to be discarded), either to clearing agent (p. 38), or to mountant (pp. 40, 43) ;
 - (b) for a stained permanent preparation (i.e. one to be kept, possibly, for a period of years), to the desired stain (p. 30).

SMEARING AND DISSOCIATING

Some tissues, e.g. blood, do not lend themselves to sectioning. Again, it may be desirable to make a preparation of fresh unhardened tissue, e.g. fresh spinal cord or contents of seminal vesicles of earthworm. In these instances the tissue, if of a sticky consistency, may be smeared *very thinly* on the centre of a slide (area of smear should be about the same as that of a cover-slip) or on a cover-slip—depending on the final technique to be employed. If more rigid it may be convenient to mount it in a drop of suitable medium (pp. 40, 43) and then, if necessary, dissociate it into smaller pieces by teasing apart with two needles, preferably of platinum (unless otherwise stated) to avoid any risk of interference by the metal with subsequent staining reactions. Methods for the preparation of certain smears are given in Chapter VI ("Methods for Specific Material") (p. 111). Otherwise the tissue may be treated as for material in very thin sections.

STAINING AND COUNTERSTAINING

Object of Staining : In dealing with the object of fixation it was remarked that in an unstained tissue the only parts distinguishable from one another were those having different refractive indices. By staining, i.e. dyeing the tissue, the phenomenon of colour can be introduced. Those parts of the cell or tissue which stain differently because of chemical and/or physical differences can thus be more easily distinguished. This is particularly helpful when different components, which, because of their like refractive indices, would remain indistinguishable, stain with different dyes, or with different intensities of the same dye, and hence become well defined.

Summarized then, one may say that the object of staining is to render parts of the tissue more obvious.

The mechanism of the process is not fully understood and hence one can do no more than summarize the tentative explanations which have been put forward.

Dyes, i.e. stains, are coloured organic substances (usually salts) capable, by their inherent properties, of being held by tissues even though treated later by the solvent in which they were themselves dissolved. From a chemical standpoint they may be classified as :

- (i) *basic*, when they consist of a coloured organic base combined with an uncoloured acetate, chloride, or sulphate radicle. They may dissolve in water and/or alcohol. Examples are safranin O, and hæmatoxylin.
- (ii) *acid*, when they consist of a metallic base, usually sodium or potassium, combined with a coloured organic acid radicle. They, also, may dissolve in water and/or alcohol. Examples are light green S.F. yellowish, and eosin Y.
- (iii) *neutral*, when they consist of the mixed watery solutions of particular acid and basic dyes. They may dissolve in water, more usually in alcohol, and often give colloidal solutions. One example is neutral red.

From the point of view of microtechnique, stains may be classified as :

- (i) *histological*, when they serve to define tissues.
- (ii) *cytological*, when they serve to define cell components.

Such stains may be sub-classified as :

- (a) *cytoplasmic*, having an affinity for the cytoplasm.
- (b) *nuclear*, having an affinity for the nucleus.

This classification is not precise, for some cytological stains serve quite well for histological purposes.

Two theories are put forward to explain what happens when a tissue is stained :

THE PHYSICAL THEORY. The stain is either,

(a) dissolved in the substance which it stains (this is undoubtedly what happens when a fat-soluble dye, such as Sudan III, is used to stain fat), or,

(b) condensed on, or attracted to the surface of the tissue, the process being known as adsorption.

THE CHEMICAL THEORY. The dye is supposed to combine chemically with some portion of the protoplasm. This gives rise to a classification of different parts of the protoplasm based on their affinities for different dyes :

(a) *basophil(ic)*, these parts, being of an acid nature, have an affinity for basic dyes. For example, the nucleus is rich in metaphosphoric acid. When treated with a basic stain the acid is supposed to combine with the coloured basic part of the dye to form a coloured insoluble salt. Hence *nuclear* stains are *basic* stains.

(b) *acidophil(ic)* or *oxyphil(ic)*, these parts, being of a basic nature, have an affinity for acid dyes. For example, the cytoplasm is usually regarded as basic. If it is treated with an acid stain the basic portion of the cell component is supposed to combine with the coloured acid radicle of the dye to form a coloured insoluble salt. Hence *cytoplasmic* stains are *acid* stains.

The terms "cytoplasmic" and "nuclear" must not be used in too restricted a sense, for basic stains, i.e. so-called "nuclear stains," always stain the cytoplasm. On the chemi-

cal theory this would be explained by saying that the acidity of the cytoplasm, though less than that of the nucleus (in which the nuclei acid combines with basic dyes), is, nevertheless, sufficient to attract basic dyes. Again, some acid stains, in addition to staining the cytoplasm, are also selective for certain nuclear elements. On the chemical theory one explanation is that the acid dye combines with the protein part of nucleoprotein.

(c) *eosinophil(ic)*, applied to acidophil granules having a particular affinity for the acid dyes eosin Y, and orange G.

(d) *neutrophil(ic)*, applied to certain cells (e.g. certain white blood corpuscles) which have an affinity for neutral dyes and which dye strongly and impartially with eosin Y (an acid dye), and methylene blue (a basic dye).

Space does not allow of a discussion of the respective merits of the physical and chemical theories. The general balance of opinion seems to favour the belief that vegetable tissues probably stain mostly by a physical process (cellulose, for example, is neither basic nor acidic), while animal tissues probably stain by a combination of both physical and chemical processes.

In the foregoing theories it has been assumed that the staining process has been *direct*, i.e. that the dye attached itself directly to the tissue. Some dyes will not do this and the *indirect* process must be used. A substance known as the *mordant* acts as a sort of intermediary between tissue and dye. The tissue is first mordanted, i.e. treated with the mordant, which incorporates itself (? combines) with some part or parts of the tissue. On the addition of the dye it is believed that the lake, i.e. the colouring matter, is carried mechanically into the mordanted tissue—probably by surface adsorption on the mordant. In some processes the tissue is first mordanted and then stained, while in others the mordant is mixed with the staining solution.

The action of some stains is helped by the addition to them of substances called *accentuators*, which act as catalysts. An example is found in Loeffler's methylene blue when potassium hydroxide—the accentuator—is added to the stain.

Some of the basic aniline dyes do not stain all the cell and

tissue components the same colour as they themselves possess. For example, safranin O stains cytoplasm in varying shades of red and cartilage matrix yellow. This phenomenon is known as *metachromasy* and should not be confused with *specificity*. Where the latter occurs, certain dyes, given specific conditions, stain only certain tissues or components, and the stain is said to be *specific* for the tissue concerned. Examples of specificity are the blue-to-black colour given to yellow elastic fibres by Weigert's elastin stain; the bright red colour given to lignin by phloroglucinol; the bright yellow given to the same tissue by aniline chloride; and the blue colour given to cellulose by Schulze's solution.

Counterstaining (Differential Staining).

The phenomenon of *selectivity* is frequently used in the processes of counterstaining, when first one part of the cell or tissue is stained with a suitable dye and the other parts are afterwards treated with a stain of contrasting colour. It should be understood that in many instances the process of counterstaining really involves the displacement of one dye by another. The dye first used (e.g. safranin O) stains all the tissues. The dye next used (e.g. Delafield's hæmatoxylin) will also stain all the tissues, but the two dyes operate together by differential displacement till, finally, for example, the lignin is stained red and the cellulose blue.

Yet another, less well known, method of *differential substitution staining* involves the use of free dye-acids and dye-bases. The principles involved are as follows: *

The acid dyes eosin Y and erythrosin bluish in their usual states are the coloured sodium salts of the acids tetrabromofluorescein and tetraiodofluorescein respectively. The dyes are soluble in water but not in most organic solvents. The free acids can be separated from an aqueous solution of the dyes by treating the aqueous solution with hydrochloric acid which combines with the sodium and leaves the free dye-acid as a solid. The free dye-acids give colourless solutions in ether or xylene. From these colourless solutions colour is

* Adapted and amended from *The New Phytologist*, 33, No. 4; 1934. R. C. Maclean.

again produced by the addition of either alkalis (e.g. sodium hydroxide gives the coloured sodium salt), or *an electronegative adsorbent, such as cellulose, with which an ionic exchange can be effected and which adsorbs free, coloured ions of tetrabromofluorescein or tetraiodofluorescein.*

(The process was originally fallaciously suggested as a test for alkali in tissues.)

The basic dyes methylene blue and Nile blue sulphate (which gives better results) may be treated in a similar manner. The free dye-base can be separated from an aqueous solution of the dyes by treating the aqueous solution with sodium hydroxide, which gives an alkali salt plus the free dye-base as a solid. The free dye-base gives a purplish-red solution in xylene. From this solution the true colour is again produced by the addition of either acids (e.g. hydrochloric acid gives the coloured acid salt of the dye), or, *an electropositive adsorbent such as lignin, which adsorbs from the xylene solution of the dye-base, free, coloured ions of the dye by ionic exchange.*

The xylene-methylene blue (or xylene-Nile blue sulphate) and the xylene-eosin Y (or xylene-erythrosin bluish) may be used for staining appropriate tissues, or they may be used consecutively in the order stated (but *not* together) for counterstaining lignin and cellulose respectively. See page 199 for technique.

Double (and Triple) Staining. These terms are, to a degree, synonymous with counterstaining but should, strictly, be applied only to the use of a mixture containing two (or three) stains of contrasting colours, which are applied simultaneously.

Methods of Staining.

The staining process may be *progressive* or *retrogressive* (*regressive*).

(a) *Progressive Staining.* This depends on the fact that certain dyes, e.g. carmine and hæmatoxylin, stain first the nucleus and then the cytoplasm. The tissue is placed in diluted stain and deliberately understained. More stain is then added gradually till the desired intensity of colour in nucleus or, later, in nucleus and cytoplasm is obtained. If sectioned

tissue is being stained, the degree of progress may be watched by placing the staining vessel on the microscope stage and examining under low power.

(b) *Retrogressive (Regressive) Staining*. The tissue is placed in a stock solution of stain, deliberately overstained and then *differentiated* or *destained*, i.e. excess stain removed by a suitable agent. The beginner will find some difficulty in judging the correct degree of differentiation and the tissue must be examined frequently under the microscope as in the progressive method. Further information will be found under "Differentiation" (p. 32).

Opinions differ, and, while the progressive method is slower, **a good rule for the beginner is to use it.**

Tissues may be stained either as sections, or smears, or in bulk.

SECTION STAINING. This is the most satisfactory method and should always be used for large pieces of tissue and for botanical work unless otherwise specified.

SMEAR STAINING. This is quite satisfactory if the smear is very thin.

BULK STAINING. This is quite satisfactory for small whole animals, e.g. protozoa, small crustacea, chick embryo, but should be avoided for large pieces of tissue and for botanical work unless otherwise specified. It is difficult to judge when the tissue is sufficiently stained and it will be found that the stain is rarely evenly distributed.

Technique of Staining.

Staining, differentiation, counterstaining, dehydration, and clearing of loose sections and of smears on cover slips, are best done in very small watch-glasses kept covered to exclude dust. In this way several sections or smears may be dealt with at once, and they are under better control than if the operations are carried out on a slide and the appropriate reagents added drop by drop.

On certain occasions (e.g. smears of blood, etc.) it may be necessary to stain the material on the slide. When such is the case and when sections attached to slides are being stained (see Chapter V, p. 95), it will be found that two 3" x 1" slides

can be placed back to back in a specimen tube $3\frac{1}{2}" \times 1\frac{1}{8}"$ (internal measurements) fitted with a cork.

It is a saving of time to have fitted, in a portable wooden rack, a series of small glass-stoppered bottles of those stains and other substances in most common use. A suggested series and sequence is: Distilled water (in a large wash-bottle); glycerine (50%) (in a bottle fitted with a cork and glass rod); borax carmine (alc.); Delafield's hæmatoxylin (aq.); eosin Y (aq.); iodine (aq.); light green S.F. yellowish (in clove oil and alcohol); safranin O (alc.); Canada balsam (in xylene) (in a bottle fitted with a domed stopper outside, and a glass rod).

Before commencing to stain, consult Chapters VI (p. 111) and VII (p. 220) to find the best stain to use for the given tissue, the specific action of the stain chosen, and the composition of the stain.

Remember that *if an alcoholic stain is used, the tissue must first be dehydrated up to the strength of alcohol used as a solvent (usually 50% or 70%).*

If, after consultation of the chapters mentioned above, there is still doubt as to the best stain to use, *the beginner should confine himself to the methods given under "Animal Tissue—general histology" (p. 117) and "Plant Tissue—general histology" (p. 194). These methods are all perfectly satisfactory for most histological work.*

It is useful to keep a written record of the results of the various methods.

Stains should be diluted 50/50 before use. Care must be taken that the stain is diluted with the solvent in which it is made up. See Chapter VII (p. 220).

The time occupied in staining, counterstaining, differentiating and washing varies with the type of tissue and its size, the type of stain, and its strength and condition.

The following may be taken as a *very rough guide*:

Staining and Counterstaining	(Sections). (Bulk).
Differentiating	(2 mins.) (1 week).
Washing	(For as long as the tissue was in the stain).
	(2 mins.) (12 hours).

Where this guide, *which is admittedly too general*, is not sufficient, explicit directions are given.

DIFFERENTIATION

Differentiation, or destaining, is carried out during the process of retrogressive staining. The purpose is, as its name implies, to differentiate the degree of staining of various parts of the cell or tissue and to remove excess stain.

The most usual differentiating agent is acid alcohol, i.e. alcohol (70%) to which 1% of acetic acid or 0.5% of concentrated nitric or hydrochloric acid has been added, but it is rather drastic and apt to diffuse the stain, and for these reasons retrogressive staining is not always to be recommended for the beginner.

When acid alcohol is used as the differentiator, it is probable that the acid of the acid alcohol has a greater affinity for the basic dye than has the cytoplasm or nucleus. As has been said, the chromatin of the nucleus is more strongly acid than the cytoplasm and consequently the nucleus parts with its dye later than does the cytoplasm. The result is a differential colouring.

Acid alcohol is not of universal use as a differentiating agent and in some instances the agent varies with the stain, e.g. clove oil is used to differentiate crystal violet; iron-alum solution (p. 256) is used to differentiate iron-hæmatoxylin.

Technique of Differentiation.

- 1° Place the tissue in a watch-glass in acid alcohol (p. 221) (unless some other differentiating agent is advised for the stain in use), *until the required density of colour is obtained*. Place the watch-glass under the low power of the microscope and observe the progress of differentiation. If sections attached to slides are being differentiated (Chapter V, p. 95) carry out the process in flat-bottomed corked specimen tubes—internal measurements $3\frac{1}{2}'' \times 1\frac{1}{8}''$.
- 2° Wash in alcohol (70%).

DEHYDRATION

Water will not mix with the resinous media in which stained and sectioned material may finally be preserved, nor with the paraffin wax with which it may be necessary to impregnate material before sectioning. The purpose of dehydration is to remove *all* traces of water from the tissue before either impregnating or finally mounting.

The dehydrating agent probably in most common use at present is ethyl alcohol, for it is miscible not only with water but also with the *solvents* used for the resinous mounting media and for paraffin wax. Unfortunately, as indicated below, alcohol is liable to distort tissues unless it is used in graded strengths, therefore the process of dehydration is slower when alcohol is used. Furthermore, ethyl alcohol is expensive; though expense may be reduced by substituting isopropyl alcohol or acetone in the last stages of the process. Latterly, other dehydrating agents have been discovered which have not these disadvantages and which may, therefore, supplant the time-honoured ethyl alcohol. One of these is ethylene glycol monoethyl ether *—commonly called “cellosolve”—which is miscible with water and xylene and is also a solvent for certain stains. Others are “Solvax” and diethylene dioxide—commonly called “dioxan” †—which are miscible with water, alcohol, and molten paraffin wax. Dioxan is reputed to have a poisonous vapour, but about this the writer has not been able to find any authentic information and he has certainly experienced no ill effects from its use for short periods on a small scale. Yet another dehydrating agent is available for use before impregnating with wax. This is butyl alcohol, which is considerably cheaper than ethyl alcohol and which, like dioxan, has the added advantage that it is miscible with paraffin wax, thus rendering clearing before wax-impregnation unnecessary.

* See *Watson's Microscope Record*, H. F. Frost, No. 34, 19; Jan. 1935; and R. H. Thorp, No. 38, 22; May 1936. “Cellosolve” is obtainable from Messrs. T. Gerrard & Co., Ltd., 46A and 48 Pentonville Road, London, N.1, and other dealers.

† See *Watson's Microscope Record*, No. 39, 22; Sept. 1936.

Technique of Dehydration.

Sections may be dehydrated in watch-glasses. (When absolute alcohol is in use the watch-glass must be kept covered.) Bulk tissue and slides with sections attached (p. 96) should be placed in flat-bottomed corked capsules—internal measurements $3\frac{1}{2}'' \times 1\frac{1}{8}''$.

METHOD A. USING ETHYL ALCOHOL

It is a saving of time to have a portable wooden rack made to carry a series of small bottles containing the following alcohols: 30% (in a larger wash-bottle); 50%; 70%; 90%; 100%. (See p. 222.)

While certain tissues may be transferred direct from (say) water to absolute alcohol without damage to their structure, this is by no means usual. Most tissues when transferred direct from water to alcohol (100%) are too rapidly plasmolysed, i.e. the water is removed from them too rapidly, and as a consequence they shrink and may be distorted. Shrinkage and distortion are avoided by gradual dehydration with strengths of alcohol gradually increased, and this should be accepted as a standard method whatever the tissue, unless an exception is specifically made.

Start the dehydration with the strength of alcohol immediately above that in which the tissue was last placed, e.g. If the tissue has been in an alcoholic stain, first consult Chapter VII, page 220, to find the strength of alcohol in which the stain was dissolved.

		(Bulk Tissue.)	(Sections.)
1°	Transfer to alcohol (30%)	(30 mins.)	(1 min.)
2°	" " " (50%)	(30 ")	(1 ")
*3°	" " " (70%)	(30 ")	(1 ")
Either,			
4°	" " " (90%)	(6 hours)	(2 ")
5°	" to fresh " (90%)	(6 ")	(2 ")
†6°	" to " (100%)	(6 ")	(2 ")
†7°	" to fresh " (100%)	(6 ")	(2 ")

*† See Notes on next page.

Or, cheaper method,	(Bulk Tissue.)	(Sections.)
4a° Transfer to industrial spirit (95%)	(6 hours)	(2 min.)
5a° " " fresh " "	(6 ")	(2 ")
6a° " " isopropyl alcohol or acetone	(6 ")	(2 ")
7a° " " fresh isopropyl alcohol or acetone	(6 ")	(2 ")

N.B.—Shake off excess isopropyl alcohol before clearing.

Notes :

* Tissues already fixed and washed may be brought to this stage of dehydration and preserved in alcohol (70%).

† When using cedar-wood oil as the clearing agent the final dehydration should be in alcohol (97%), and when using benzene-phenol or clove oil the final dehydration may be in alcohol (96%) containing 5% phenol.

Absolute alcohol is very expensive and it is more economical and quicker to do the final dehydration in two successive changes of small volumes of alcohol rather than in one large volume. For the first of these changes the alcohol from the *last* change of a *recent final* dehydration may be used.

The bottle of absolute alcohol will not remain absolute if you breathe or sneeze into it. The dehydration with alcohol (100%) must be done in a small closed vessel. When transferring sections to alcohol (100%) make sure that the camel-hair brush is not impregnated with alcohol weaker than 100%.

Remember that the dehydration—as well as the final alcohol—must be absolute ! * Slackness here—as shown by a whitish film when the tissue is subsequently immersed in benzene or xylene (if either of these be used)—spells complete disappointment later.

METHOD B. USING BUTYL ALCOHOL

Refer to permanent preparation method PW-1 (wax-embedded sections) and proceed with the processes from 5° under Method B, p. 101.

* See remarks under "Clearing," p. 37.

METHOD C. USING "CELLOSOLVE" *

(Ethylene glycol monoethyl ether) (Ethoxy-ethyl alcohol)

Cellosolve is miscible with water, with alcohol and with xylene. As it does not distort thin pieces of tissue there is no need to grade the dehydration, nor is there any necessity to clear the tissue. It should *not* be used for dehydration of material in bulk.

Material for dehydration is transferred direct from water or from any strength of alcohol (if, for example, it has been preserved in alcohol, or if it has been in an alcoholic stain) into cellosolve. Dehydration of sections takes about one minute and they may then be mounted immediately in Canada balsam. Cellosolve is also miscible with clove oil so that material dehydrated in cellosolve may be transferred direct to a clove oil solution of a stain such as would be used in the safranin O and light green S.F. yellowish method mentioned on page 86.

The method of using cellosolve when tissue is to be wax-impregnated will be found on page 102.

METHOD D. USING "DIOXAN"

(Diethylene dioxide)

Refer to permanent preparation method PW-1 (wax-embedded sections) and proceed with the processes from 5° under Method D, page 103.

METHOD E. USING "SOLVAX"

Refer to permanent preparation method PW-1 (wax-embedded sections) and proceed with the processes from 5° under Method E, page 104.

CLEARING

Although alcohol is miscible with the solvents both for the paraffin wax used for impregnating and for the resinous media used for mounting, it is not miscible with either paraffin wax or the resinous media themselves. In addition, the alcohol will have lowered the refractive index of the tissue.

* See p. 33 for footnote.

The object of clearing is to remove alcohol prior to impregnating or mounting, and, indeed, the process is sometimes called dealcoholization. It is called clearing because, when the tissue is thoroughly soaked with the clearing agent, its refractive index is raised and, as previously explained, it becomes more transparent (see "Refractive Indices," pp. 277, 278). Transparency is an indication that clearing is complete and the subsequent process should not be started till this state is reached.

The clearing agent used will depend on the general method adopted for the preparation. For example, isotonic saline solution is suitable for temporary preparations of animal tissue, while eau de Javelle, or chloral hydrate, are useful for temporary preparations of plant tissue.

For permanent preparations of both animal and plant tissues a very commonly used clearing agent is xylene, but it has two serious disadvantages. One, that it tends to shrink and harden tissues; the other, that if the tissue to be cleared has not been completely dehydrated the water present forms a whitish-looking temporary emulsion with the xylene. Though the presence of this emulsion may (and should) be taken as a warning sign of incomplete dehydration (in which instance dehydration must be continued) it is best avoided if possible. If no other clearing agent is available, and xylene must be used, then, in every 100 c.c. xylene dissolve 5 grams of phenol crystals.

There are other, and better, clearing agents than xylene. Baker (*Cytological Technique*, Methuen) strongly recommends benzene. This does not shrink and harden the tissue to the same extent as xylene but, unfortunately, it also emulsifies with water. Baker avoids this, and the high cost of absolute alcohol, by doing the last dehydration in alcohol (96%) containing 5% phenol, and adding 5% phenol to the benzene.

For the best work on animal tissue it is advisable to use cedar-wood oil. (When ordering, state that it is required for clearing and not for use with oil-immersion lenses. The lower price of the former variety is a consideration.) Cedar-wood oil, though clearing more slowly than xylene or benzene,

is the least likely to shrink or harden the tissue. It mixes only in certain proportions and with certain strengths of alcohol. Baker recommends final dehydration in alcohol (97%) and transference of the tissue to a 50/50 mixture of cedar-wood oil/absolute alcohol and then to pure cedar-wood oil for equal periods. There is no limit to the time during which tissues may be left in cedar-wood oil. Sections cleared in cedar-wood oil tend to retain drops of the oil when mounted, and this should be removed by quickly passing sections through xylene prior to mounting.

For the best work on plant tissues use clove oil as the clearing agent. Clove oil also has its disadvantages. It is liable to make objects brittle. It removes many stains, e.g. hæmatoxylin, crystal violet, orange G, and safranin O, and when used for clearing sections stained with those dyes it should be followed quickly by cedar-wood oil. As explained under cedar-wood oil, the sections cleared in clove oil should be washed rapidly in xylene to remove oil drops. One advantage of clove oil is that sections may be transferred to it from alcohol (95%) or even (90%), and another, that it is useful when the nature of the material necessitates the sections being thick.

The last remark also applies to another clearing agent, lacto-phenol, which has the additional advantage of being useful for clearing the more delicate algæ and fungi (see pp. 113, 163, 259).

Berlese's fluid may be used for clearing small whole insects (see pp. 170, 229).

Technique of Clearing.

Clearing of sections, smears on cover-slips, and small pieces of tissue can be done in a watch-glass. Bulk tissue and sections attached to slides should be treated in flat-bottomed corked capsules—internal measurements, $3\frac{1}{2}" \times 1\frac{1}{8}"$.

Before using any clearing agent, see notes on Refractive Indices (p. 277).

A. Temporary Preparations of Animal Tissues.

Mount in a saline (isotonic salt) solution (p. 281).

B. Temporary Preparations of Plant Tissues.

(a) 1° Eau de Javelle (p. 243). (2-3 mins.).

2° Wash with distilled water.

3° Wash with dilute acetic acid.

or (b) Chloral hydrate (p. 235). (Overnight).

C. Permanent Preparations of Animal and Plant Tissues. (*Small whole objects, bulk tissue, and sectioned tissue.*)

Having decided on the clearing agent *according to the recommendations given above*, make up the following mixtures (which may be used again and again) in specimen tubes.

MIXTURE I. "25/75."

*Clearing agent 25 c.c.

Alcohol (100%) 75 c.c.

MIXTURE II. "50/50."

*Clearing agent 50 c.c.

Alcohol (100%) 50 c.c.

MIXTURE III. "75/25."

*Clearing agent 75 c.c.

Alcohol (100%) 25 c.c.

then : (Bulk Tissue.) (Sections.)

[See Note (d).]

*1° Transfer to mixture I (25/75) (24 hours) (2 mins.).

*2° " " " II (50/50) (") (").

*3° " " " III (75/25) (") (").

4° " " pure clearing agent (Till transparent)

Note :

(a) As the clearing agent varies so may the final dehydration.

*(b) When using cedar-wood oil do not shake it with the alcohol. Gently add the alcohol to the oil and then, likewise gently, add the tissue. As the tissue is penetrated by the oil it will sink.

*(c) When using cedar-wood oil, 1° and 3°, and in urgent work by beginners, 1°, 2° and 3°, may be omitted.

(d) The time for clearing bulk tissues varies with the clearing agent and the tissue and its size. Twenty-four hours may be

considered a maximum. Half an hour may be sufficient. If xylene is used, make the time as short as is consistent with complete clearing.

(e) See Chapter VI (p. 111) for special methods for clearing algæ, fungi, insects, crustacea.

MOUNTING AND COVERING

The purpose of mounting the tissue is to enable it to be examined under the microscope. The carrier for the tissue is a thin glass slide about $3'' \times 1''$. The medium in which the tissue is mounted varies with the type of preparation.

If the preparation is for temporary use, then the mountant is liquid to prevent the tissue drying, e.g. distilled water, saline solution, or glycerine. (See p. 43.)

If the preparation is to be of a permanent nature, then the medium is usually resinous or gelatinous for preservation, e.g. Canada balsam or glycerine-jelly. (See p. 44.)

After immersion in the mounting medium, the tissue is covered with a thin glass cover-slip to exclude air and dust.

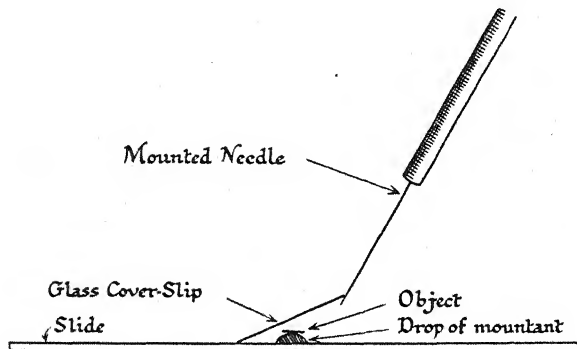
Technique of Mounting and Covering.

The plane glass slides in most general use measure $3'' \times 1''$. The object should be mounted in the middle of the slide. When the cover-slip has settled on the mountant, the latter should spread just to the edges of the slip—no less—no more. There may be a little difficulty in judging the correct quantity of mountant to ensure this. Perfection will come with practice; at first the beginner should use circular cover-slips ($\frac{3}{4}''$ diam.), and then try $\frac{3}{4}''$ square slips. For long specimens use rectangular slips $1\frac{1}{2}'' \times \frac{7}{8}''$. Until really proficient, use slips No. 3, 0.21–0.35 m.m. in thickness, to avoid excess breakages. Afterwards use slips No. 1, 0.17–0.18 m.m. in thickness (see Chapter III, pp. 54, 60).

- 1° Place a drop of mountant (see p. 43) on a *clean* slide (p. 249). It is well to give the slide and cover-slip a

final polish with an old—but clean—linen handkerchief kept for the purpose. To clean the cover-slip, hold opposite edges between the finger and thumb of one hand and polish it very gently with the handkerchief held between finger and thumb of the free hand.

- 2° By means of a small camel-hair brush place the object in the mountant.
- 3° Place one edge of a *clean* cover-slip on the slide to one side of the mountant, gently lower it by means of a mounted needle, and *allow it to settle itself* (Fig. 1).



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FIG. 1.—Method of lowering cover-slip on to object and mountant.

If, by bad manipulation, air bubbles are included, warm the slide *very* gently over a small, non-luminous, gas flame.

The position of minute objects on the slide may be indicated by applying to the cover-slip a ring of Indian ink surrounding the position. The ink is best applied with the thinnest sable brush obtainable, or else by a fine mapping pen, while the slide is revolved on a turntable (see "Ringing," p. 47).

Note :

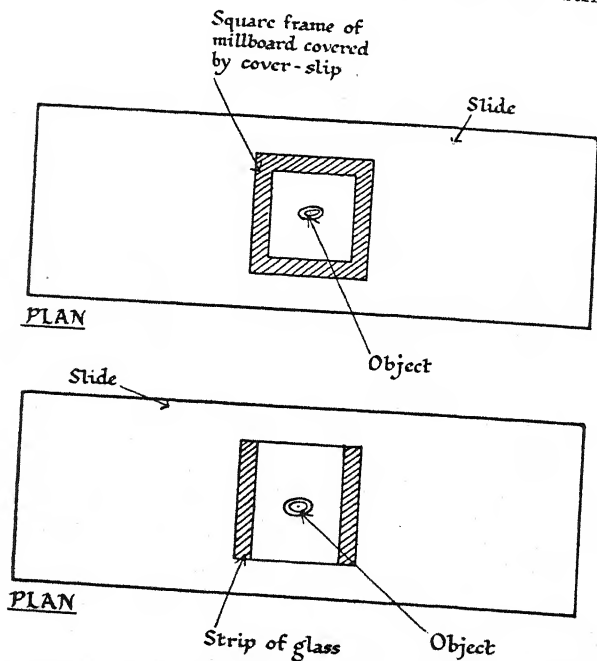
If the object is thick and in danger of being crushed, raise the cover-slip off the slide, thus :

- (i) Cut a square or circular frame of mill-board or very

42 OUTLINES OF PRINCIPLES AND TECHNIQUE

thick paper of the same shape and area as the cover-slip and mount the object in the space thus enclosed (Fig. 2). Or, better,

(ii) Cut strips of glass from a slide or a cover-slip the same length as the width of slip to be used. Place two strips in



FIGS. 2 AND 3.—Devices for raising cover-slip above a thick preparation. [J. G. Hawkes.]

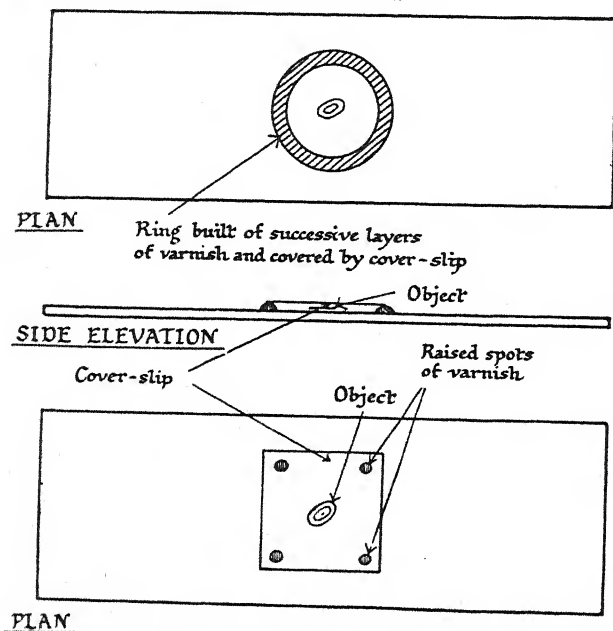
position corresponding with the ends of the cover-slip. Mount the object between them (Fig. 3).

Or,

(iii) Build up a series of superimposed rings (p. 48) of varnish (p. 278) by successive applications and dryings on the slide. Mount the object inside the ring (Figs. 4 and 5).

Or,

(iv) Build up four raised spots of varnish to fit just inside the four corners of a cover-slip placed over them (Fig. 6). Mount the object between the four spots.



[J. G. Hawkes.

FIGS. 4, 5, AND 6.—Devices for raising cover-slip above a thick preparation.

Or,

(v) Use a ring of sheet aluminium, 0.003" thick. Before use, flatten by pressing between two slides.

Mountants.

I. TEMPORARY MOUNTANTS.

A. ANIMAL TISSUE.

- (i) Saline (isotonic salt) solution (p. 281).
- (ii) Distilled water.

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(iii) *Glycerine.*

1° Wash in distilled water.

2° Mount in glycerine (p. 250).

B. PLANT TISSUE.

(i) *Distilled water.*

(ii) *Alcohol (30%).*

(iii) *Glycerine.*

1° Wash in distilled water.

2° Mount in glycerine (p. 250).

II. PERMANENT MOUNTANTS FOR BOTH ANIMAL AND PLANT TISSUE.

(i) *Glycerine.*

(a) Useful for delicate objects which might be injured by too much manipulation. *There is no need to dehydrate or clear.*

1° Wash object in distilled water.

2° Drain.

3° Transfer either, (i) to glycerine (10%),
or, (ii) to a solution of

Distilled water 5 gr.

Industrial spirits 4 gr.

Glycerine 1 gr.

4° Allow the glycerine or the solution to concentrate by evaporation at room temperature. *(Several days.)*

5° Transfer the object to a drop of pure glycerine on a cover-slip.

6° Invert the cover-slip on to a slide provided with four legs of varnish, or cement, or tea-chest foil punched into discs 1-2 m.m. in diameter (Fig. 6, p. 43).

7° Coat the margin of the cover-slip (which will not be touched by the glycerine) with paraffin wax.

8° By means of a hot wire, melt the wax and run it under the slip to fill the space

between the mountant and the margin of the slip.

9° When the wax has set, remove the excess with a warmed, narrow, carpenter-type chisel.

10° Ring with shellac varnish (pp. 47, 278).

(b) For large objects.

1° Wash object in distilled water.

2° Drain.

3° Mount in glycerine (p. 250).

4° Cover.

5° Ring (p. 47) cover-slip with gold size (p. 250).

(ii) *Glycerine jelly.*

Remarks as for glycerine; rather easier to use.

1° Wash object in distilled water.

2° Drain, and soak object in glycerine (40%).
(3 mins.).

3° Melt the glycerine jelly (p. 250) on a water-bath.

4° Place object on cover-slip.

5° Feed the fluid jelly on to the object with a warm pipette.

6° Invert cover-slip on to a warmed slide.

7° Ring (p. 47) cover-slip with gold size (p. 250).

This method obviates the use of small cubes of jelly which easily allows of overheating of the jelly and of entrapping of air bubbles.

(iii) *Farrants' medium.*

Remarks as for glycerine jelly, but more useful because there is no need to ring the cover-slip.

1° Wash object in distilled water.

2° Drain thoroughly.

3° Soak object in Farrants' medium (p. 246).

4° Breathe on a slide and add the specimen.

5° Add a drop of Farrants' medium to the specimen.

6° Breathe on a cover-slip and lower it, damp side downwards, on to the specimen. (The dampness prevents bubbles forming in the medium.)

(iv) *Canada balsam in xylene* (p. 232).

The most generally useful medium.

Canada balsam is best kept in wide-mouthed bottles (painted black outside to prevent ingress of light and consequent acidification of the balsam) containing a small glass rod and supplied with a domed glass stopper which fits outside the neck of the bottle. Avoid cork stoppers; the cork disintegrates and fouls the balsam. If the balsam thickens, dilute it slightly with the appropriate solvent (either xylene or benzene). On pouring out fresh Canada balsam always wipe the inside of the neck and the stopper of the stock bottle with a lint-free cloth soaked in xylene. This prevents the stopper sticking and obviates much annoyance later. When removing the rod bearing the drop of balsam for mounting, be careful to lift it clear of the neck of the bottle to prevent fouling.

Note : Special methods for mounting protozoa, coelenterates, platyhelminthes, crustacea, insects, small algæ, fungi, etc., are given in Chapter VI (p. 111).

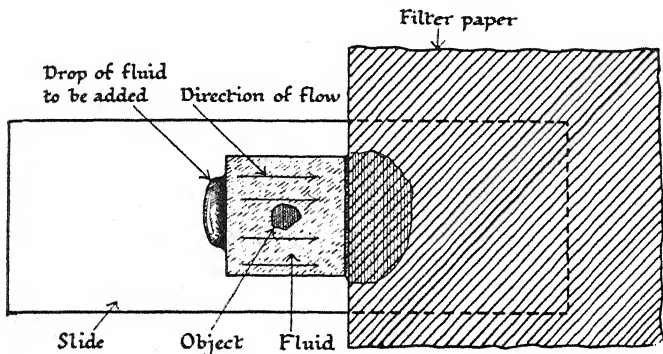
IRRIGATION

It is sometimes necessary, especially when dealing with small animals and plants, whether live or dead, to treat them with fluids while on a slide under the cover-slip. This is accomplished by *irrigation*.

It is assumed that the object is already immersed in some fluid, and this must reach at least to two opposite sides of the cover-slip. The fluid to be introduced is placed in a drop at

one edge of the cover-slip. A piece of filter paper (a useful size, cut ready for convenient handling, is 2" \times 3") is placed with one edge against the opposite side of the cover-slip. The filter paper draws out the liquid already under the slip, and the fluid to be added flows in to take its place (Fig. 7).

Irrigation should always be done gently to avoid washing the specimen out.



[J. G. Hawkes.]

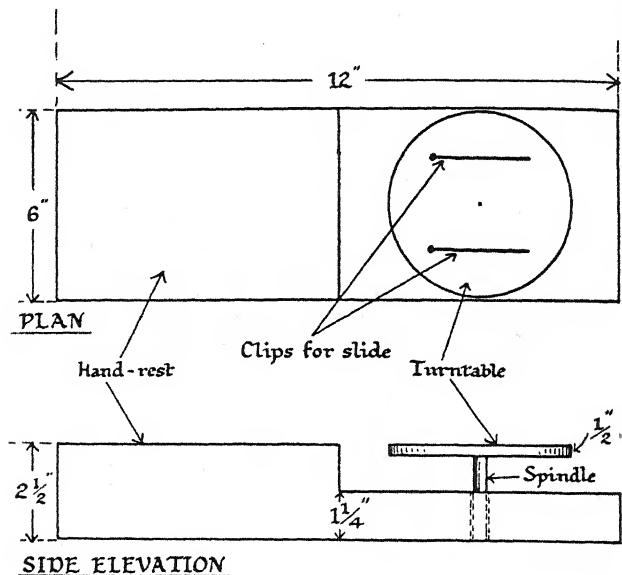
FIG. 7.—Irrigation under a cover-slip.

RINGING

In course of time the liquid mountants and the solvents of the resinous mountants evaporate, and it sometimes happens that a tissue cannot well be preserved permanently in a resinous medium. In such instances it is necessary to mount in a more liquid medium and to run a ring of cement or varnish round the edge of the cover-slip to exclude air and prevent evaporation of the mountant. This process is known as *ringing* and is sometimes used even for Canada balsam mounts when it is desired to give a particularly fine finish to a slide. Ringing media include, amongst others, cellulose varnish, Brunswick black, gold size, and Canada balsam. Others are mentioned under "Cement" (p. 234) and "Ringing Media" (p. 278).

Technique of Ringing.

The ringing medium, which should be fairly stiff, may be carried on a No. 2 best-quality sable brush and applied to the edges of the cover-slip. If the cover-slip be circular, this



[J. G. Hawkes.

FIGS. 8 AND 9.—Arrangement of turntable for ringing preparations.

is more easily done by holding the slide by clips on a simple rotating turntable as shown above (Figs. 8 and 9).

Apply a *thin* ring first, wait for the material to dry, and ring again. Gradually build up a thick ring in this way. If the rings become wide or uneven, use the point of a pen-knife blade to push the ring of medium where it is required while the turntable is revolving at a good speed.

LABELLING

Labelling of Slides.

After mounting and covering the tissue, the slide should be labelled, preferably in Indian ink, with :

- (1) the name of the tissue and location of the section ;
- (2) the methods of fixation and staining (this is important for interpreting subsequent examination) ;
- (3) the date of preparation ;
- (4) the maker's name.

Use labels with a good adhesive—bad stickers are a source of annoyance in a dry atmosphere. Labels should be about $1" \times \frac{3}{4}"$ and placed at one end of the slide. If the slide is to be stored on its long edge, the writing on the label should be parallel with the long side of the slide. If it is to be stored flat, the writing on the label should be at right-angles to the long side. In damp atmospheres it is wise to protect written labels by brushing them over with a very thin coating of molten paraffin wax.

Labelling of Capsules.

Capsules—flat-bottomed specimen tubes used for carrying tissue in bulk and sections attached to slides should be labelled :

- (a) outside, to indicate the liquid they contain,
- (b) inside, to indicate the tissues under treatment. This is best done in pencil writing on a piece of plain white paper slipped inside the capsule.

PREPARATIONS FROM WAX-EMBEDDED TISSUE

The techniques of all the processes for making preparations of wax-embedded tissue are given in Chapter V, page 88.

CHAPTER III

THE MICROSCOPE AND ITS USE

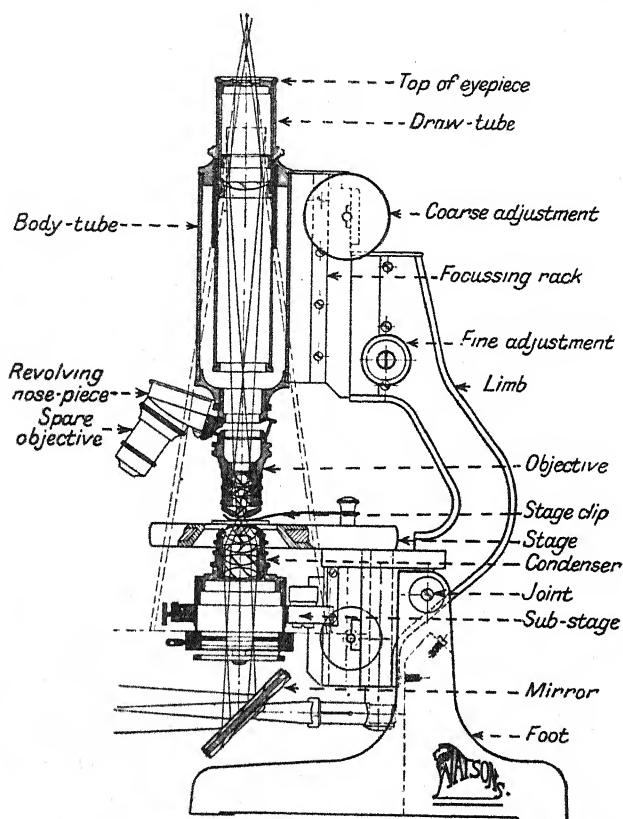
Construction of the Compound Microscope

The essential parts are indicated in Figs. 10 and 11. The main body of the microscope is known as the stand and consists of a base or foot to which is attached a limb. The limb is generally made inclinable (by means of a joint) relatively to the foot. At its lower end the limb carries a mirror mounted on a gymbal so that its position may be varied with the source of light. The mirror usually has two sides, one plane, the other concave. Above the mirror there may be an iris diaphragm for controlling the amount of light reaching the object. Above the diaphragm is the stage which has, at its centre, a circular aperture for the passage of light. The stage may be of the "mechanical" variety which enables delicate adjustments of the position of the object to be made. Inserted in holes on the stage are stage clips for clamping in position the glass slide carrying the object.

At its upper end the limb carries a body-tube within which there may be a draw-tube. These tubes are blackened on the inside to prevent reflection. At the lower end of the body-tube is a nose-piece into which is screwed a fitting carrying a lens, or series of lenses, called the objective. The nose-piece may be double (or triple) and carry two (or three) objectives in such a manner that they may be revolved into position (and approximate focus) without the necessity of unscrewing the objective previously in use.

Into the upper end of the body-tube (or draw-tube, if this be present) there slides another fitting carrying a lens (or series of lenses) called the ocular or eyepiece.

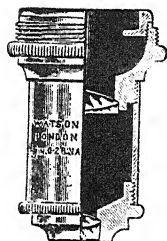
Fitted in a suitable carrier below the stage there may be a system of lenses called the sub-stage condenser. This carrier can also be fitted with a ring to hold discs of coloured glass



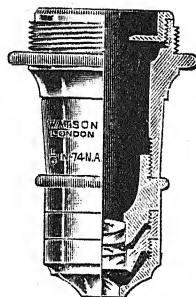
[Reproduced by permission of Messrs. W. Watson & Sons, Ltd.]

FIG. 10.

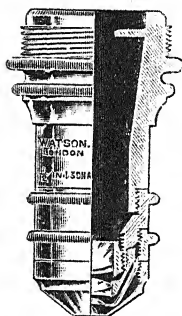
(to lessen the strain of bright light upon the eye), or discs of metal known as patch-stops (for use in dark-ground illumination).



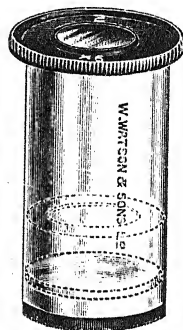
Low-power Objective
2 in. to $\frac{1}{2}$ in.



High-power Objective
 $\frac{1}{8}$ in. to $\frac{1}{4}$ in.



Oil-Immersion Objective
 $\frac{1}{2}$ in.



Eye-piece.

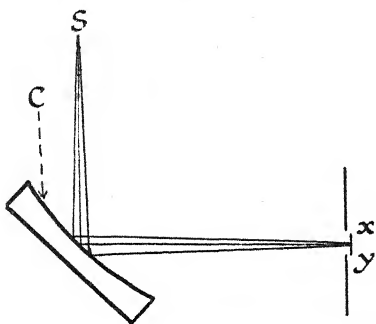
[Reproduced by permission of MESSRS. W. Watson & Sons, Ltd.
FIG. 11.—Objectives and eye-piece.

ILLUMINATION.

The effective working of the objective depends on the proper illumination of the object under examination. This may be accomplished by :

- (a) *Transmitted Light*, when the rays of light are transmitted through the transparent parts of the object by a suitable mirror. The purpose of a plane mirror (P) (Fig. 14) is to reflect the light from any given source (S) towards the object (xy). The concave mirror (C)

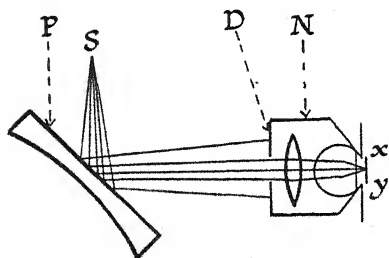
(Fig. 12) is used to concentrate the reflected light on the object (xy). The sub-stage condenser (N) (Fig. 13), in conjunction with the plane mirror P, is also used for



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FIG. 12.*—Use of concave mirror.

concentrating the light. The use of the sub-stage iris diaphragm (D) for diminishing the light rays is sometimes an aid to resolution, i.e. structure differentiation.



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FIG. 13.*—Use of plane mirror and condenser.

(b) *Reflected Light*, when the light is directed (and, if desired, concentrated by a plano-convex lens) from

* In Figures 12, 13, and 16 the mirror and object are, for the sake of comparison, shown in the same relative positions as they occupy in Fig. 14. It will be understood that in actual practice the beam of light from the mirror will be projected *upwards* to the object lying above.

above on to the object which is placed on a dark ground. This is a useful method for opaque objects.

- (c) *Dark Ground or Oblique Illumination by Refracted and Diffracted Light*, when the object is made visible by the diffraction of light rays around it, just as the particles of dust are seen in the ray of sunlight in a darkened room. The method is useful for the examination of diatoms and bacteria, and some suggestions for its use are given on p. 65.

TUBE-LENGTH AND COVER-SLIP THICKNESS.

Tube-length is the distance between the upper end of the objective and the upper end of the eye-piece. An error in tube-length gives decreased definition. Generally speaking, the objectives likely to be used by students are those corrected for use with a tube-length of 160 m.m., though this figure will vary with the thickness of the cover-slip in use. (See p. 60.)

OBJECTIVES.

Though the beginner may have his objectives ready chosen for him, it is well to have some notion of the ideas underlying the choice.

In the first instance this will depend on the class of work to be undertaken, but for general use those described as achromatic are suitable.

- (a) *Working Distance*. This is the distance between the object and the lower end of the objective. It is desirable that the objective should have as long a working distance as possible, and $\frac{1}{8}$ " objectives are now obtainable with a working distance of 1 m.m.

- (b) *Numerical Aperture*. Ability to resolve (differentiate structure) is associated with the numerical aperture (N.A.) of the lens. (This is an optical constant of the lens, and those interested might well refer to Martin and Johnson's *Practical Microscopy*.) The greater the numerical aperture, the greater the resolution. For general work, if the $\frac{1}{8}$ " objective has N.A. 0.70 it will give sufficient resolution.

- (c) *Oil-immersion Lenses.* High-power ($\frac{1}{7}$ ", $\frac{1}{8}$ ", $\frac{1}{12}$ ", and $\frac{1}{15}$ ") objectives are, with some exceptions, of the "oil-immersion" (or "water-immersion") type, i.e. a layer of cedar-wood oil (or water-soluble immersion medium) is placed between top of cover-slip and bottom of objective. This arrangement gives brighter and more perfectly magnified images.

The Compound Microscope as an Optical Instrument.

The essential optical elements of the compound microscope are the objective and the eyepiece. The instrument works as follows :

The object (xy) (Fig. 14) is placed just beyond the focal plane (f) of the objective (O), a convex lens of very short focal length ($fO, f'O$). The objective causes a real, enlarged, inverted image ($y'x'$) of the object to be brought very near the eye (i) and just within the focal plane (F) of the ocular (e), a convex lens of focal length ($Fe, F'e$) usually greater than that of the objective. The position of $y'x'$ is too near the eye (principal foci F_2, F_2') to give a clearly defined image on the retina (R). It is, therefore, viewed through the ocular which gives at $y''x''$ (the "near point," i.e. the point at the minimum distance from the eye for distinct vision) a virtual, enlarged image of $y'x'$, and one which subtends, for all practical purposes, the same visual angle [the angle subtended at the centre of the lens (L) of the eye] as $y'x'$ itself. Although the image of $y''x''$ on the retina (R) is the same size as that which would be given by $y'x'$, the former is, because of its position at the "near point," clearly defined, whereas that of $y'x'$ is not. It should be noted that, strictly, the ocular does not magnify the enlarged inverted image, $y'x'$, formed by the objective, but replaces it by $y''x''$ at such a distance that the eye can focus it.

MAGNIFICATION.

Meaning of Magnification. For the sake of comparison the magnifications may be considered relative to the apparent size of objects held 25 cms. from the eye, i.e. the least possible distance compatible with distinct vision. For

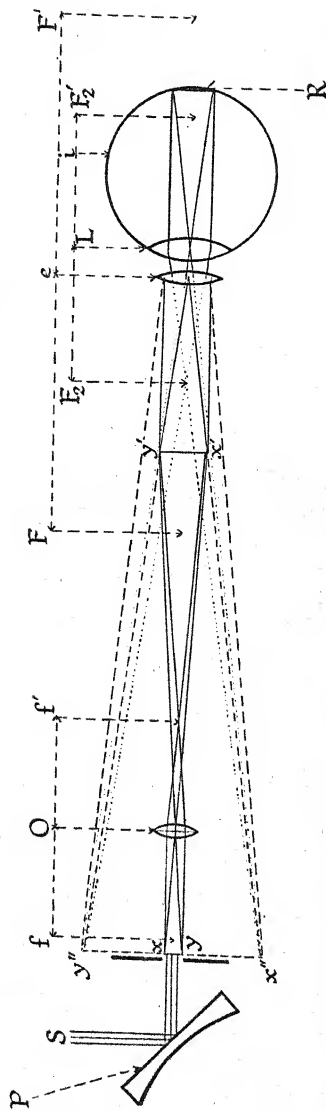


FIG. 14.—Diagrammatic arrangement of microscope and eye to illustrate positions of real, virtual, and retinal images of the object. For explanation see text, p. 55.

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e , eye-piece or ocular; f , focal length of objective; $fO, f'O$, focal length of objective; F, F' , focal plane of objective; F_e, F'_e , focal plane of ocular; i , eye; L , lens of eye; O , objective; O' , objective; P , plane mirror; R , retina of eye; S , source of light; xy , object; $y'x'$, real enlarged, inverted image of object; y, x' , virtual, enlarged image of y, x' .

example, an object of diameter $\frac{1}{10}$ m.m., if viewed through a $\times 6$ eye-piece and a $\frac{1}{8}$ " objective (a combination giving a total magnification of 240), will appear as large as a similar object $\frac{1}{10}$ m.m. $\times 240 = 24.0$ m.m. in diameter seen from a distance of 25 cms. away from the eye.

The Microscope in Use

CLEANING.

It is a good plan never to tamper with the microscope, but it is impossible to see well with dirty lenses. These must never be cleaned with a handkerchief, or scratched lenses will result. Always use a piece of best quality chamois leather. (There should be a piece allocated to, and kept in, each microscope case.) If the dirt is obstinate the leather may be moistened with alcohol (100%). If, through what can only be sheer carelessness, some Canada balsam has found its way on to the objective, wipe it off gently with the leather moistened with benzene, and finish off with a dry chamois leather.

Neglected lenses sometimes acquire a greasy deposit. Remove this with a trace of clean paraffin oil applied with a piece of the softest tissue paper. Polish off gently with a clean piece of tissue paper.

Do *not* use the lens leather, but an old linen handkerchief for wiping the stage.

ADJUSTMENT OF TRANSMITTED LIGHT.

Insert a low-power objective ($1''$ or $\frac{2}{3}''$) and adjust the mirror to give the maximum amount of light in the centre of the field of view. This may be better judged by first removing the eye-piece.

- (i) *Daylight.* When working in daylight, the direct rays of the sun should not be used. Light from cloud in the north sky is desirable. Use the concave side of the mirror normally. With some delicate objects better resolution may sometimes be obtained by adjusting the mirror so that the light is not fully concentrated on the object. It may even be desirable in such circumstances to use the plane mirror—the

worker must experiment for himself. When the condenser is in position, the plane mirror must be used.

- (ii) *Artificial Light.* If electric light is available use an opal or frosted bulb behind a blue glass screen. The drawback to plain bulbs is that the filaments are reflected. If a frosted bulb cannot be obtained, use a "daylight" bulb placed behind a ground glass or tracing-paper screen. If only a plain white bulb is obtainable let the screen be of blue glass with tissue-paper pasted over it. The screen may, of course, be arranged as the front part of a box made to carry the lamp-holder. [For the amateur a very useful electric microscope lamp may be made by fixing a pocket-lamp bulb-holder in a bakelite shaving-stick case mounted to swivel on a suitable retainer and connected to a switch and battery.] If electricity is not available, incandescent gas or an oil-lamp must be used.

Whether the plane or concave side of the mirror is used in artificial light will depend on the nature of the object and the power of the objective. Although, as previously indicated, the concave side will give the greater concentration of light, it may be found that the light is too intense (with *possible* consequent loss of detail) unless the sub-stage iris diaphragm is used to cut off some of the light. If no diaphragm is fitted it is best to start with the plane side of the mirror. When the condenser is in position the plane side of the mirror must be used.

ADJUSTMENT OF REFLECTED LIGHT.

For the illumination of opaque objects, light is focussed on to the object from an oblique angle. This is accomplished by means of a plano-convex lens, i.e. a "bull's eye." The lens is mounted on a stand provided with a ball-and-socket joint so that it may be adjusted to any desired angle between illuminant and object.

Whether the plane or the convex side of the lens is turned

towards the object will depend to some degree on the particular lens and on the source of light. The author's experience has been that both in artificial light and in daylight a better result has been obtained by turning the *plane* side towards the object. This may be because the source of light is coming from an extended surface and, when the plane side of the lens is turned towards the object, both surfaces of the lens take part in producing deviation.

* Illumination is increased if a piece of thin white card is placed underneath the slide carrying the object. Cut a piece of postcard $5'' \times 1''$, and make slight transverse folds $1''$ from each end. The centre portion of $3''$ will carry a slide and the $1''$ portions at the ends will form "handles." When examining white or pale objects, replace the white card by a black one; or place a piece of black paper between white card and slide.

When very small opaque objects are being examined with high-power lenses having short working distances, the source of light must necessarily be almost on a level with the microscope stage, and the light must be re-focussed on to the object by some form of parabolic reflector. Work of this kind is not likely to be done by the elementary student; those interested should refer to some advanced book on microscopy for details of the apparatus and technique involved.

ADJUSTMENT OF DARK GROUND ILLUMINATION.

Separate instructions will be found on page 65.

ADJUSTMENT OF OBJECT.

Remember that *all* microscope lenses have a curved field and that the focus is absolutely sharp only in the centre of the field.

Place the slide, object upwards, on the stage and secure it by means of the stage clips. The slide should be adjusted so that the object is directly underneath the low-power objective already in position. The presence of the object in the field of view will be recognized by the decrease in illumination and

* Adapted from *The Structure and Development of the Fungi*, Gwynne-Vaughan and Barnes, Cambridge University Press.

by the appearance of colour if the object be coloured. The eye-piece, if previously removed, should now be replaced.

COVER-SLIP THICKNESS AND ADJUSTMENT OF TUBE-LENGTH.

A cover-slip of correct thickness should be used, but without the maker's catalogue for guidance this dimension may not be known. In general, a cover-slip of thickness 0.17 m.m. or 0.18 m.m. will be correct. If the cover-slip is thicker than 0.18 m.m., decrease the tube-length; if thinner than 0.18 m.m., increase the tube-length.

Remember that increase of depth of burial in mountant is equivalent to increase of cover-slip thickness.

CHOICE OF LENS COMBINATIONS.

The higher the power of the lens and the larger its numerical aperture, the more spherical the field. The lower the magnification, the flatter the field.

As a guide to the beginner, the following combinations* of objectives and eye-pieces may be taken as suitable for the purposes indicated. Those in brackets are desirable additions. See page 63 for the magnifications obtained from various combinations.

Botany, Zoology, and General Biology . . .	Objective. [2"], $\frac{2}{3}$ ", $\frac{1}{6}$ ".	Eye-piece. [$\times 6$], $\times 8$, [$\times 10$].
Wing of fly; scale of fish (for detail use $\frac{2}{3}$ " obj.); sting of bee; fruits of dandelion, clematis, and birch . . .	2".	$\times 6$.
Rotifera . . .	[3"], 2".	[$\times 6$], $\times 8$, [$\times 10$].
Circulation in tadpole tail; water flea; scales of small fish . . .	1", [$\frac{2}{3}$ "].	$\times 6$.
Foraminifera . . .	2", 1".	[$\times 6$], $\times 8$, [$\times 10$].
Chætæ of earthworm; barbules of feather; hairs on fruits of dandelion, etc. . .	$\frac{1}{4}$ ".	$\times 8$.
Control of staining . . .	$\frac{2}{3}$ ", $\frac{1}{6}$ ".	$\times 6$.

* In part, after Shann, *First Lessons in Practical Biology*, Bell.

	<i>Objective.</i>	<i>Eye-piece.</i>
Epidermis of leaf; pollen grains; blood corpuscles; unicellular animals from hay infusions	$\frac{1}{8}"$.	$\times 10$.
Animal and plant histology .	$\frac{2}{3}"$, $\frac{1}{8}"$.	[$\times 6$], $\times 8$, [$\times 10$].
Spermatozoa of earthworm; blood corpuscles; yeast; <i>Pleurococcus</i> ; bacteria (appear as specks) . . .	$\frac{1}{8}"$.	$\times 15$.
Bacteriology; hæmatology; pathology; serology . . .	$\frac{2}{3}"$, $\frac{1}{8}"$, $\frac{1}{12}"$.	[$\times 6$], $\times 8$, [$\times 10$].

Note: The low-power ($\frac{1}{4}"$ to $\frac{1}{2}"$) and the medium-power ($\frac{1}{4}"$ to $\frac{1}{8}"$) objectives are, with occasional exceptions, of the "dry" type, i.e. there is a film of air between top of cover-slip and bottom of objective.

ADJUSTMENT OF FOCUS.

(i) *Low-power.* Place the head to one side of the instrument and, by means of the coarse focussing device, bring the *low-power* objective down fairly close to the object. Now, using one eye (whichever is found to be the more convenient) *and keeping the other eye open*, bring the object into focus by racking *upwards*, i.e. by using the coarse focussing device to draw the objective away from the object. This lessens the risk of cover-slips being cracked and of object and objective being ruined by the objective being crashed on the slide. Using low-power, the focus obtained with the coarse adjustment will probably be sufficiently sharp.

The low-power (L.P.) gives a general view and may be used for searching. *It is a good habit always to use the L.P. first*, and make a drawing of the main features of the object on the same scale as they appear under the microscope.

The microscope field may be represented on the drawing-paper by a circle drawn with the handiest guide available—usually a 4.5 cm. diam. watch-glass.

Mark on the drawing the magnification represented. [See "Magnification" and "Measurement" (pp. 63-5).]

(ii) *High-power.* If it is desired to see one particular part

of the object under H.P., first bring this part into the centre of the field under L.P.

Great care must be exercised in focussing the $\frac{1}{8}$ " and higher-power, objectives. This is best accomplished by using the fine adjustment to bring the objective so close to the cover-slip that, when the eye is placed on a level with the stage, the objective appears almost to touch its reflection in the cover-slip. The objective may now be drawn away from the stage by means of the fine adjustment, and the object will come into focus.

Most modern microscopes, when fitted with revolving nose-pieces, are so designed that once the object has been focussed through one objective it is almost in focus when another objective is brought into position. But remember that an extra thick cover-slip or a ring of cement may project so high as to catch the base of a high-power objective as it is swung into position. When this is likely to occur the objective must be racked up beforehand.

Keep both your eyes open, move gently, and never use any force.

The high-power (H.P.) gives a detailed view. Do not attempt to make a drawing of the whole field but only of representative areas. The position of the chosen areas should be indicated on the drawing from L.P.

(iii) *Oil Immersion Objectives.* First centre the object under a medium-power objective. Change over to a $\frac{1}{8}$ " or $\frac{1}{1\frac{1}{2}}$ " objective. Now place one drop of cedar-wood oil, or one drop of golden syrup diluted with its own volume of water, either on the cover-slip, dead central over the object, or on the objective. (It is better to use golden syrup for preparations which might be damaged by the use of xylene when the cedar-wood oil is being cleaned off.) Next lower the objective with the usual, or even greater care. Great care really is necessary (especially in moving the slide), and the thinnest cover-slips must be used.

After use, the oil is carefully removed from the objective and slide by means of a chamois leather soaked in xylene. The xylene may be removed by alcohol, as indicated in the paragraph on cleaning. Golden syrup is removed by water.

MAGNIFICATION.

- (a) *Formula for finding magnification.* Total magnification (in diameters) of objective and eye-piece

$$= \frac{l}{f} \times e.$$

Magnification of objective

$$= \frac{l}{f}$$

l = tube-length, i.e. the distance between the upper end of the objective and the upper end of the eye-piece.

f = focal length of objective (as marked on the objective).

e = magnification of eye-piece (as marked on the eye-piece), i.e. the number of times the eye-piece will magnify the image produced by the objective.

- (b) *Table of approximate magnifications.**

Focal Length of Objective = f	Magnification of Eye-piece = e						
	No. 0 × 4	No. 1 × 5	No. 2 × 6	No. 3 × 8	No. 4 × 10	No. 5 × 12	No. 6 × 15
2" or 50 m.m. . .	13	16	19	26	32	38	48
1" or 25 m.m. . .	26	32	38	51	64	76	96
3" or 16 m.m. . .	40	50	60	80	100	120	150
1" or 8 m.m. . .	80	100	120	160	200	240	300
1" or 6 m.m. . .	106	133	160	213	266	319	399
1" or 4 m.m. . .	160	200	240	320	400	480	600
1" or 3 m.m. . .	212	265	318	424	530	636	795
1" or 2 m.m. . .	320	400	480	640	800	960	1200
1" or 1.7 m.m. . .	376	470	564	752	940	1128	1410
Tube-length = l = 160 m.m.	Approximate Total Magnification = $\frac{l}{f} \times e$						

Heavy type indicates combinations frequently used in elementary work.

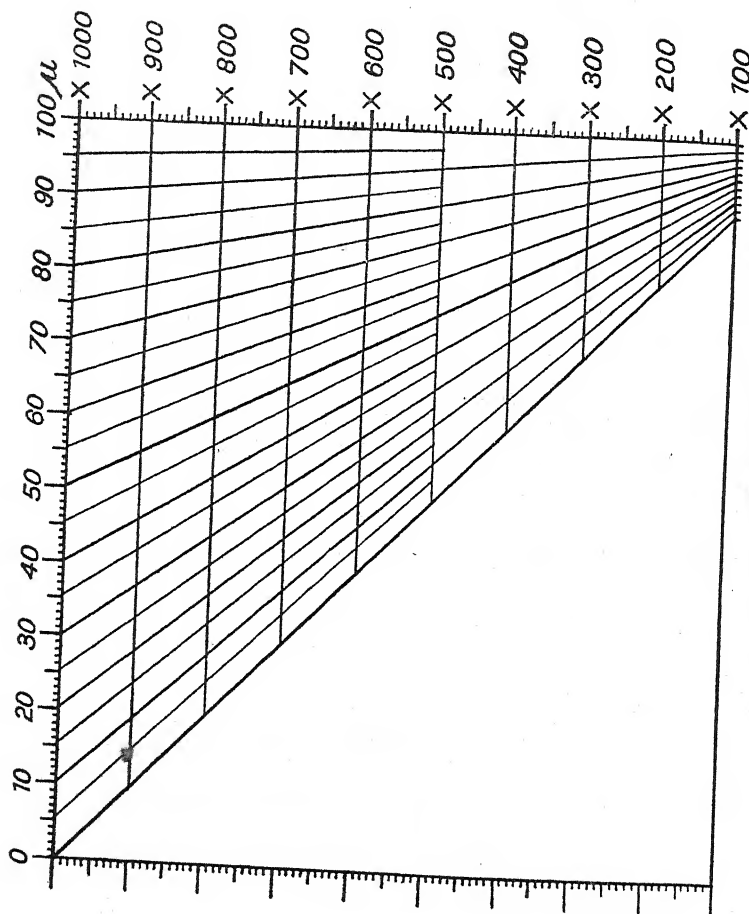
MEASUREMENT.

- (a) *Microscopic measurement.*

The unit of microscopic measurement is 0.001 m.m.

$$= 1\mu = 1 \text{ micron} = \frac{1}{25000}'' \text{ (approx.)}$$

* Adapted from Martin and Johnson's *Practical Microscopy*, by permission of the publishers, Messrs. Blackie.



[Reproduced by permission of Messrs. W. Watson & Sons, Ltd.
Fig. 15.—The micrometric rule.

- (b) *The Micrometric Rule—actual size of objects seen under the microscope.*

Without some basis of comparison it is difficult to appreciate the actual size of the object one sees magnified, or reproduced in a photomicrograph. A suitable basis is afforded by the Micrometric Rule * shown on the previous page.

The vertical scale on the right represents magnifications. If the apparent size of the object † at any given magnification be marked off on the appropriate horizontal line at the points of intersection with the diverging lines, its actual size in microns will be indicated by the top horizontal scale. Thus, suppose at magnification $\times 100$ an object appears to be 1 m.m. long, its actual size = 10μ (1 m.m. = $100 \times 10 \times 0.001$ m.m.).

When drawing an object as seen through the microscope, always state the magnification. The micrometric rule may then be used to copy on to the drawing the given scale representing 100μ for the magnification in use.

For magnifications ten times those given, the divisions will represent values one-tenth of those indicated. For magnifications one-tenth of those given, the values represent ten times those indicated.

DARK-GROUND ILLUMINATION.

The technique of dark-ground illumination is not difficult to acquire, but it should not be attempted until the student has become thoroughly acquainted with the use of the microscope with transmitted light.

- (a) *The following will be required :*

A sub-stage condenser with a numerical aperture about 1.0. (The N.A. will be found marked on the lens.) A sub-stage

* Devised independently by F. Addey (*Quekett Mic. Club Journal*, Nov. 1921), and by J. A. Lord (*Watson's Microscope Record*, May 1934).

† i.e. the size of the image. As seen in the microscope, this is determined by a stage micrometer. See Bibliography—"Masters and Johnson."

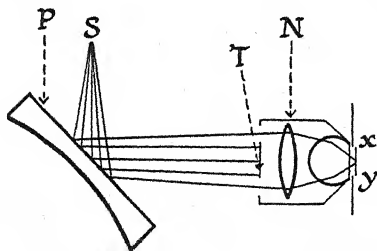
diaphragm with stop-carrier. A set of patch-stops ranging in diameter from about 2.3 cms. to 0.75 cm. These may be made from celluloid discs (cut to fit the stop-carrier) by painting circular patches of Brunswick black on them. A $\frac{2}{3}$ " objective with N.A. about 0.28, and a $\frac{1}{4}$ " objective with N.A. less than 0.6. Eye-pieces $\times 8$ and $\times 10$.

(b) *Preliminary focussing.*

Arrange a fairly small diaphragm aperture and, with a twisting motion (unless there be a device for focussing the sub-stage condenser, when the normal mechanism will be used), lower the condenser (N, Fig. 16) so that its upper end is a little below the base of the slide. Focus the object (*xy*) under the $\frac{2}{3}$ " objective (the position of the condenser may require adjustment) and arrange the light *dead central* in the field. Use as bright a light(s) as close as possible to the plane side of the mirror (P). If the white light is irritating, insert a coloured glass on the stop carrier.

(c) *Obtaining the dark ground.*

Now insert a moderately small patch stop (T) on the stop carrier (first removing the coloured glass if this was



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FIG. 16.*—Use of patch-stop and condenser for dark-ground illumination.

inserted). Open the diaphragm as wide as possible, taking care not to move the position of illuminant or objective. Carefully move the condenser upwards (twisting motion) until

* See footnote to Fig. 12, p. 53.

it is almost touching the base of the slide. The object will now be illuminated obliquely. Focus may be adjusted if necessary.

(d) *Use of high-power objective.*

Change over to the $\frac{1}{4}$ " objective and $\times 10$ eye-piece. Replace the patch-stop by a larger one (about 2 cms. diam.) and adjust the focus. It should not be necessary to adjust the condenser. It is possible, with this combination of lenses, to see the rolling motion of spirochaetes from the teeth.

(e) *Additional points.*

The lower the power of the objective, the smaller the patch-stop required.

The higher the power of the objective, the bigger the patch-stop required.

Too small a patch-stop gives an indistinct field, and too big a patch-stop gives no contrast.

Eye-pieces of too low power result in the field being incompletely darkened.

Different eye-pieces require different patch-stops.

Chromatic aberration (the image being surrounded by coloured fringes) and spherical aberration (the image being indistinct) cannot be avoided with thick objects. Clarity will be greatest at the centre of the field.

The best combinations of objectives, eye-pieces, patch-stops and condenser positions will be found by experiment.

If the only high-power objective available has too high a N.A. (for example, a $\frac{1}{8}$ " objective often has N.A. 0.85), remove the lens at the base of the objective, place on top of it a metal washer (suitably blackened), such as is used on electric terminals, and replace the objective. The correct size of washer must be determined by experiment.

Slides and cover-slips must be clean and thin, or definition will be poor. No. 1 cover-slips (0.17 m.m. thick) should be used.

When changing from dark-ground to transmitted light, rack the condenser down.

(f) *Suitable material for practice.*

Remove some of the deposit on the teeth with a matchstick. Smear it on a clean, thin slide. Cover with a No. 1 cover-slip.

Simple Microscope Devices.

SIGHTING-LINE FOR MICROSCOPE.*

- 1° Obtain a single strand of artificial silk.
- 2° Place a drop of gold size on diametrically opposite sides of the diaphragm of the microscope eye-piece.
- 3° Spread the gold size in a thin film by means of a match and allow it to dry.
- 4° By means of forceps place one end of the strand of silk in the gold size at one side and stretch the silk across the diaphragm to the opposite side, keeping it taut by pressing it radially, with the end of the match, in the gold size.

When fixed, the silk should be taut and straight across the middle of the diaphragm, in focus of the eye-piece, and appear as a thin dark line on the slide.

CROSS-WIRES FOR MICROSCOPE.†

- 1° Fix two (non-greasy) human hairs at right-angles on a piece of paper by means of gummed labels.
- 2° Place a circular cover-slip ($\frac{3}{4}$ " diam.) under the hairs so that the centre of the cover-slip is under the point of intersection of the hairs.
- 3° Place a drop of fairly stiff, warm Canada balsam on the hairs at the point of intersection.
- 4° Cover with another slip, taking the usual precautions as for mounting.
- 5° Allow to dry. When quite dry cut the hairs at the edges of the slip by means of a really sharp scalpel.
- 6° Remove eye-piece from microscope and place the cross-wire device on the stage below the eye-piece. Replace the eye-piece.

* After Meakin, *Watson's Microscope Record*; No. 32, 14; May 1934.

† Adapted from Ashby, *School Science Review*, Vol. XV, 1934. Murray.

STAGE MICROMETER.* (*For finding cell or stomata dimensions.*)

- (i) 1° On a piece of tin-foil outline an area the size of a cover-slip.
 - 2° Place two thin glass slides parallel with each other on a piece of glass so that the distance between them just exceeds the diameter of a very fine sewing needle.
 - 3° Stretch the tin-foil across the slides so that the marked area is over the gap.
 - 4° With a very fine sewing needle punch about 36 holes in the tin-foil, distributed evenly over the marked area, using the gap between the slides as a guide, and the glass beneath as a stop.
 - 5° By means of a vernier microscope measure the diameter of the holes (it will be found that they are fairly constant) and calculate the average area of each hole. If the same apparatus is used it should not be necessary to measure the holes in each foil prepared. Meanwhile, prepare a suitable storage receptacle and label it with the average hole-area of the batch of foils being made.
 - 6° Cut round the marked area of perforated foil.
 - 7° Choose a suitable temporary mountant and mount the piece of tissue to be examined on a slide.
 - 8° Cover with the perforated foil.
 - 9° Cover the whole with a cover-slip of the same shape and area as the foil.
 - 10° Count the average number of (cells) visible per hole and calculate from the data now available
 - (1) The number of (cells) per sq. m.m.
 - (2) The diameter of the (cells).
- (ii) See also "Measurement" (pp. 63-5).

COVER FOR MICROSCOPE.†

A cover made of thick "Cellophane," of ample size and suitable shape, is useful when it is desired to leave the micro-

* After Ridley, *Science Masters' Book*, Part II, Murray, London.

† After Harris, *Watson's Microscope Record*, No. 33, 23; Sept. 1934.

scope out of its normal case or cupboard for a short length of time. Gum arabic, or glue with 15% glycerine added, are suitable adhesives for "Cellophane."

SCREEN FOR MICROPROJECTOR.

Where it is not possible to darken a room really efficiently, reasonably good results can be got from a microprojector, in a moderately well-darkened room, if the image is viewed from behind a translucent screen, i.e. the screen is between viewer and microprojector.

Suitable materials for such a screen are the translucent "Cellophane"-like substances called "Bexoid" (clear matt/matt)* and "Celastoid"†; and the "Illusion Fabric Screen."‡

The material can be stretched over a wooden frame of size to suit the demonstrator's requirements.

TEST FOR EFFICIENCY OF MICROPROJECTOR.

For really effective *class* demonstration work, a microprojector should be able to give a clearly defined image of cell-division in *Rhabditis* (see p. 205), provided the microscopic preparation has been well done. For such a demonstration the microprojector must have a $\frac{1}{6}$ " objective, and the object must be well illuminated by a lamp having a condensed filament. Suitable illumination can be obtained from a 60-watt motor-car headlamp. For work of this sort the microprojector must be water-cooled.

* Obtainable from The British Xylonite Company, Hale End, London, E.4.

† Obtainable from Messrs. Newton & Co., 72 Wigmore Street, London, W.1.

‡ Obtainable from Messrs. J. B. Kleinertz, 25 Newgate Street, London, E.C.1.

CHAPTER IV

GRADED METHODS FOR MAKING MICROSCOPICAL PREPARATIONS OF ANIMAL AND PLANT TISSUES

"... It is nevertheless a depressing fact that one could be good at stabbing a liver with a cork-borer, and bad at making cytological preparations."

J. BRONTË GATENBY.*

By microtechnique is meant the making of preparations of plant and animal tissue suitable for examination through a microscope.

The preparation may be of a temporary or permanent nature.

By a temporary preparation is meant one which, by its nature, will not remain suitable for microscopic examination after a comparatively short time, perhaps an hour or even less.

By a permanent preparation is meant one which can be stored, and which will remain suitable for microscopic examination during a long period of years. Even some permanent preparations are liable to deteriorate.

What to do. If asked to "make a microscopic examination of the tissue provided," it may be assumed that a temporary preparation is wanted.

In any case, it is always advisable to make such a preparation before proceeding to any specialized technique. Remember that "the capacity for making full use of unstained sections and, in fact, the direct observation of living material are the foundation of biological training. . . ."†

* *Nature*, 134, 477; Sept. 29, 1934.

† Godwin, *New Phytologist*, XXXIV, No. 3; 1935.

If a permanent preparation is required, this fact will usually be stated, but, even so, it is better first to make a temporary preparation and then, if the piece of tissue in use is worth preserving, to embark, with a new piece of the same tissue, on one or other of the methods for making permanent preparations.

In this chapter some common methods for making temporary and permanent preparations of plant and animal tissue are outlined. The number of tissues which it is suggested the beginner should use has been purposely limited so that confidence may be acquired in a small field. Variations of these methods for specific material are given in Chapter VI (p. 111). Methods for wax embedding and for bulk staining of tissues are given in Chapter V (p. 88).

The beginner is recommended,

(1) to work through the methods in this chapter in the order in which they are given ;

(2) to make a point of referring to the appropriate section of Chapter II (p. 11) before attempting any process. In that chapter will be found a more detailed explanation both of the principles underlying each process and of the technique of carrying it out with a good chance of success. Without a knowledge of the underlying principles one is necessarily working in the dark, and his work is bound to be slipshod and the final result poor ;

(3) to master the technique of each process (explained in detail in Chapter II, and merely outlined below) and get really good results from each method before passing on to the next ;

(4) to make sure that his glass-ware is clean (see p. 249) ;

(5) to read Chapter III (p. 50) so that he may learn something about the microscope and its use before attempting to use it ;

(6) to see that his razor is sharp before attempting to cut sections (see p. 275) ;

(7) to exercise care in every process.

PRELIMINARY EXERCISES

Before making any microscopic preparations it is best to become acquainted with the microscopic appearance of "foreign bodies":

- (a) Examine a dirty slide under low-power and high-power objectives. Dirty glass-ware and dirty lenses tend to give cloudy and "fuzzy" images.
- (b) Place, in turn, some dust, a few sand grains, fibres of wool, cotton, linen, and silk on a clean slide. Examine and draw under a low-power objective. Do not examine under a high-power objective because the objective is likely to be damaged unless the objects are covered with a cover-slip.
- (c) Note the microscopic appearance of air-bubbles. By means of a glass tube drawn out to a jet of suitable size (to be determined by trial and error), place a drop of water or glycerine in the middle of a clean slide. Mount a small quantity of any of the materials mentioned in (b) (above) in the mountant. Cover with a clean cover-slip according to the technique given on page 40. The mountant should spread just to the edges of the cover-slip—no less and no more. At the first attempt air-bubbles will almost certainly be included, due either to bad judgment of the quantity of mountant required, or to bad manipulation of the cover-slip, or both. Examine and draw the bubbles under low power and the objects under high power. Note the difference in appearance of the objects when mounted in a liquid to exclude air.

In future, regard the inclusion of any dirt, foreign bodies, or bubbles as a piece of bad workmanship.

TEMPORARY PREPARATIONS.

METHOD T-1. Unstained Whole Mount

Material suggested: Colonial alga; piece of onion skin;
hay infusion.

Process

Comment

1° Mount the material
in water.

(p. 40.) Place a drop of water in the middle of a glass slide. By means of a mounted needle or a pointed camel-hair brush transfer some of the material to the water. Purpose is to keep the material from drying during examination.

2° Cover.

(p. 40.) Place a thin glass cover-slip over the object. Purpose is to prevent both evaporation of the mountant and damage to the microscope objective. This process is normally assumed to be included in the mounting process.

TEMPORARY PREPARATIONS.

METHOD T-2. Irrigated Whole Mount

Suggested material: Colonial alga; epidermis of onion leaf; hay infusion.

<i>Process</i>	<i>Comment</i>
1° Mount in water.	(p. 40.)
2° Cover.	(p. 40.)
3° Irrigate with dilute iodine.	(p. 46.) Using the same piece of material as in Method T-1 the purpose is to observe changes wrought by the irrigant. With iodine, starch granules will be stained blue; protoplasm brown; protozoa will be killed; cilia will be made plain.

TEMPORARY PREPARATIONS.

METHOD T-3. Unstained Sections

Suggested material: Transverse sections of carrot.

*Process**Comment*

1° Section.

(p. 23.) Purpose is to have tissue so thin that light reflected from microscope mirror will pass through tissue and microscope lenses to observer's eye. Cut very thin transverse sections by a razor, freehand. Do not stop at one section; cut several at a time and do not reject thin sectors. By means of a clean small quill-mounted camel-hair brush transfer the sections from the razor to a watch-glass of distilled water.

2° Mount in glycerine.

(p. 40.) Place a drop of glycerine (50%) in the middle of a slide. With the camel-hair brush transfer the thinnest section to the drop of mountant.

3° Cover.

(p. 40.)

TEMPORARY PREPARATIONS.

METHOD T-4. Unstained Smear

Suggested material: Squamous epithelium.

*Process**Comment*

- 1° By means of a blunt clean scalpel scrape some epithelial cells from the inside of the cheek.
- 2° Smear some of the cells on the middle of a slide* (p. 24).
Smear should be thin (to show individual cells) and occupy about the same area as a cover-slip.
- 3° Add a drop of isotonic (mammalian) saline solution (p. 281) to the smear.
This mountant prevents the cells from shrinking or swelling.
- 4° Cover.

* If preferred, the tissue may be smeared on a cover-slip, which would then be inverted in a drop of saline previously placed on the slide.

TEMPORARY PREPARATIONS.

METHOD T-5. Irrigated Smear

Suggested material: Squamous epithelial cells from cheek.

*Process**Comment*

- 1° Smear the tissue on a slide.* Thinly, in the middle (p. 24).
- 2° Add a drop of isotonic (malian) saline (p. 281).
- 3° Cover. (p. 40.)
- 4° Irrigate with 1% acetic acid. (p. 46.) This irrigant will render the nuclei more easily visible.

* See footnote to Method T-4 (p. 77).

TEMPORARY PREPARATIONS.

METHOD T-6. Stained Sections

Suggested material: Transverse sections of sunflower
(*Helianthus*) stem.

Process	Comment
1° Fix the tissue.	(p. 12.) Place the tissue in (70%) alcohol for a few days. (This may have been done for you beforehand.) Purpose is to retain the tissue in as life-like a condition as possible and to harden it so that it may be sectioned more easily. Wash in fresh alcohol (70%) after fixing.
2° Wash in distilled water.	(p. 14.) Very necessary. Purpose is to remove fixative which might otherwise interfere with staining by aqueous stain.
3° Section.	(p. 23.)
4° Stain with Delafield's hæmatoxylin. (3-10 mins.).	(p. 25 f.) Purpose is to render certain parts of the tissue more visible. Choose the thinnest sections, even though they may be only sectors of the stem, and transfer them to a watch-glass containing some stock solution of stain diluted about 1 in 3 with distilled water. After 3 minutes place some of the sections on a slide and examine under the microscope to see if they are stained sufficiently. The cell nuclei will be stained deeply; the cytoplasm less deeply. If not stained sufficiently, replace in stain and try again.
5° Wash in distilled water.	To remove excess stain.
6° Wash in tap-water.	To "blue" the hæmatoxylin. If tap-water is very soft use "Tap-water substitute" (p. 285). The nuclei will be stained blue.
7° Mount in glycerine.	(p. 40.)
8° Cover.	(p. 40.)

TEMPORARY PREPARATIONS.

METHOD T-7. Counterstained Sections

Suggested material: Transverse sections of sunflower
(*Helianthus*) stem.

<i>Process</i>	<i>Comment</i>
1° Fix the tissue.	As T-6, 1° (p. 79).
2° Wash in distilled water.	As T-6, 2° (p. 79).
3° Section.	(p. 23.)
4° Stain with Delafield's hæmatoxylin. (3-10 mins.).	(p. 25 f.) Dilute the stain about 1 to 3 with distilled water. Check progress under microscope.
5° Differentiate in acid alcohol. (<i>Momentarily</i>).	(p. 32.) Purpose is to remove the hæmatoxylin from the cytoplasm which, otherwise, would give a purplish-red colour when counterstained with eosin Y.
6° Wash in tap-water.	To blue the hæmatoxylin. See T-6, 6° (p. 79).
7° Counterstain with aqueous eosin Y. (30-60 secs.).	(p. 28.) Place the hæmatoxylin-stained sections in a watch-glass of aqueous eosin Y. Purpose is to stain cytoplasm pink.
8° Rinse in tap-water quickly.	
9° Mount in 50% glycerine.	
10° Cover.	

PERMANENT PREPARATIONS.

METHOD P-1. Stained Sections

Suggested material : Transverse sections of Sunflower
(*Helianthus*) stem.

Process	Comment
1° Fix.	(p. 12.)
2° Wash in distilled water.	(p. 14.)
3° Section.	(p. 23.)
4° Stain in Delafield's hæmatoxylin. (3-10 mins.).	(p. 25.) Use diluted stain and watch progress.
5° Wash in distilled water.	
6° Wash in tap-water.	
7° Dehydrate in alcohol (30%).	(p. 33.) Purpose is to remove
8° Dehydrate in alcohol (50%).	all the water before mounting in
9° Dehydrate in alcohol (70%).	a resinous medium, with which
10° Dehydrate in alcohol (90%).	water will not mix. This is done
11° Dehydrate in alcohol (100%).	gradually to prevent shrinkage
12° Dehydrate in fresh alcohol (100%).	of tissue. Continue each stage
	for 2 minutes. Cover the vessels
	of absolute (100%) alcohol with
	another watch-glass to keep out
	water-vapour.
13° Clear in benzene-phenol or xylene-phenol. (2 mins. or till transparent).	(p. 36.) Purpose is to remove
	alcohol, which will not mix with
14° Mount in Canada balsam.	the resinous mountant.
	(p. 40.) This resinous medium
	seals the preparation and keeps
	it air-tight.
15° Cover.	(p. 40.)
16° Label.	Stick a small white label at one
	end of the slide. On it write the
	name of the tissue ; the method
	of staining ; the date of prepara-
	tion ; and your initials (p. 49).
17° Ring (if desired).	(p. 47.) This involves running
	a ring of cementing material or
	varnish round the edge of the
	cover-slip. It is not essential
	but adds to the permanence of
	the preparation.

PERMANENT PREPARATIONS.

METHOD P-2. Stained Smear

Suggested material: Squamous epithelial cells from cheek.

<i>Process</i>	<i>Comment</i>
1° Smear the tissue on a slide, thinly.	(p. 24).
2° Wave the slide in the air until the tissue is almost dry.	The tissue will stick to the slide, aided by saliva from mouth.
3° Fix with "corrosive-acetic." (2 mins.).	This fixative is very poisonous. Let a drop of fixative cover the smear. (pp. 12, 18).
4° Wash 3-4 times with distilled water.	Run distilled water on slide very gently, or tissue will be lost.
5° Stain in Delafield's hæmatoxylin. (3-10 mins.).	(p. 25.) Place sufficient stain (diluted 1 in 3 with distilled water) on the slide to cover the smear. Watch progress under microscope.
6° Wash in distilled water.	Run stain off slide and run water on gently.
7° Wash in tap-water.	Gently. To blue the stain.
8° Dehydrate in alcohol (30%).	(p. 33.) 1 minute in each.
9° Dehydrate in alcohol (50%).	Run one liquid off the slide and
10° Dehydrate in alcohol (70%).	add just enough of the next to
11° Dehydrate in alcohol (90%).	cover the smear.
12° Dehydrate in alcohol (100%).	
13° Dehydrate in fresh alcohol (100%). (1 min.).	
14° Clear in benzene-phenol. (Till transparent).	(p. 36.)
15° Mount in Canada balsam.	(p. 40.) Place a drop of Canada balsam on the smear.
16° Cover.	(p. 40.)
17° Label.	(p. 49.)
18° Ring if desired.	(p. 47.)

PERMANENT PREPARATIONS.

METHOD P-3. Stained Whole Mount

Suggested material: *Daphnia* or *Cyclops*.

For this Method first make a container, suitable for holding a number of the animals, by tying a piece of fine-mesh silk over one end of a short piece of glass tubing having about $\frac{3}{8}$ " bore. Processes 1° to 9° inclusive are carried out by dipping the covered end of the tube in each liquid (placed, for convenience, in short wide specimen tubes) to a depth sufficient to cover the animals. When each process is complete, the tube is removed, allowed to drain for a moment or two, and then placed in the next liquid.

Process	Comment
1° Kill and fix in Bouin's fluid (pp. 17, 231). (10 mins.).	The picric acid in the fixative will also stain the chitin yellow.
2° Wash in alcohol (50%). (2 mins.).	
3° Dehydrate in alcohol (70%). (2 mins.).	It is proposed to use an alcoholic stain, i.e. a stain dissolved in (70%) alcohol. Therefore dehydrate to this stage before staining.
4° Stain in borax carmine. (2 mins.).	
5° Wash in alcohol (70%). (2 mins.).	
6° Dehydrate in alcohol (90%). (2 mins.).	
7° Dehydrate in alcohol (100%). (2 mins.).	
8° Clear in clove oil or cedar-wood oil. (Till transparent).	Clove oil and cedar-wood oil can be used to de-alcoholize, even though a trace of water is present. Do not throw away the oil after use; it can be used again. Transfer it to a special bottle. Quickly. Purpose is to remove traces of oil which would show as globules under the microscope.
9° Wash in xylene.	

*Process**Comment*

10° Mount in Canada balsam.

(p. 40.) Transfer contents of tube to a watch-glass and remove animals one by one to the mountant on the slide.

11° Cover.

(p. 40.)

12° Label.

(p. 49.)

13° Ring if desired.

(p. 47.)

PERMANENT PREPARATIONS.

METHOD P-4. Counterstained Smear

Suggested material: Squamous epithelial cells from cheek.

N.B. Throughout the processes 2°-11° inclusive hold the cover-slip in a nick in a match-stick, and place it tissue downwards in the various liquids.

*Process**Comment*

- | | |
|--|---|
| 1° Smear the tissue on a clean cover-slip. | Thinly. |
| 2° Air-dry by waving the slip in the air. | Do not let the tissue dry completely. |
| 3° Fix in "corrosive-acetic."
(2 mins.). | Poison—care! Place the cover-slip, tissue downwards, in watch-glass of fixative. |
| 4° Wash in dilute iodine; and then in distilled water.
(3-4 times). | Gently, or tissue will be lost. The mercury compound must be removed. |
| 5° Stain in Delafield's hæmatoxylin.
(3-10 mins.). | Dilute the stain about 1 to 3 with distilled water. |
| 6° Differentiate in acid alcohol.
(Momentarily). | |
| 7° Wash in tap-water. | |
| 8° Counterstain in aqueous eosin Y.
(30-60 secs.). | |
| 9° Wash in tap-water. | |
| 10° Dehydrate in alcohol (96%).
(1 min.). | Although it is usual to up-grade the alcohols more gradually, the dehydration is here done more quickly because eosin Y washes out very easily in alcohol—especially alcohol (70%). |
| 11° Dehydrate in alcohol (100%).
In covered vessel. (1 min.). | |
| 12° Clear in benzene-phenol.
(Till transparent). | |
| 13° Mount in Canada balsam. | Invert the cover-slip tissue downwards on to a drop of balsam placed ready on the slide. |
| 14° Cover. | |
| 15° Label. | |
| 16° Ring if desired. | |

PERMANENT PREPARATIONS.

METHOD P-5. Counterstained Sections

Suggested material: Transverse sections of Sunflower
(*Helianthus*) stem.

<i>Process</i>	<i>Comment</i>
1° Fix.	(p. 12.)
2° Wash in distilled water.	(p. 12.)
3° Section.	(p. 23.)
4° Dehydrate in alcohol (30%). (2 mins.).	(p. 33.) It is proposed to use an alcoholic stain dissolved in
5° Dehydrate in alcohol (50%). (2 mins.).	(50%) alcohol. Therefore dehy- drate up to this point before staining.
6° Stain in safranin O (p. 280). (5 mins.).	This will stain the lignified tissue red.
7° Wash in alcohol (50%). (1 min.).	
8° Dehydrate in alcohol (70%). (1 min.).	
9° Dehydrate in alcohol (90%). (1 min.).	
10° Dehydrate in alcohol (100%). (1 min.).	
11° Counterstain in light green S.F. yellowish (in clove oil). (2 mins.).	(p. 260.) This will stain the cellulose green.
12° Wash in alcohol (100%). In covered vessel. ($\frac{1}{2}$ min.).	
13° Clear in clove oil. (2 mins.).	
14° Wash in xylene.	(Quickly). To remove drops of oil.
15° Mount in Canada balsam.	(p. 40.)
16° Cover.	(p. 40.)
17° Label.	(p. 49.)
18° Ring if desired.	(p. 47.)

Results of staining by this Method: Cellulose \rightarrow green,
i.e. all cells with cellulose walls, e.g. cortical cells of stem and
root; medullary cells of stem and root; parenchyma of
xylem and phloem; sieve tubes and companion cells in

phloem; mesophyllary cells of leaf, including parenchyma and collenchyma. Lignin → red, i.e. fibres; store cells; pericycle fibres; epidermal tissues opposite the veins in many broad leaves; hypodermal tissues in *Pinus*.

CHAPTER V

METHODS FOR WAX-EMBEDDING

Reasonably thin sections of plant tissue may often be cut with a razor, free-hand. Animal tissues, being less rigid, rarely lend themselves to this method. For the finest work with both animal and plant tissue it is necessary to cut the thinnest of sections (not thicker than about 12μ) with a mechanical microtome. For this purpose, and also when a hand-microtome is in use, the tissue, after certain preliminary treatment, is impregnated with, and then embedded in a suitable stiffening material, or else it is frozen rigid. The usual impregnating materials are paraffin wax and celloidin (purified nitro-cellulose). The freezing and celloidin methods are not likely to be required by, and hold no special advantage for, the beginner and hence will not be described here.

In the preparation of wax-embedded sections two methods are employed. Either the material—after suitable treatment—is impregnated with wax and then sections are cut and stained, or, the material is first stained in bulk and then embedded and sectioned. Whichever method is employed, once the material is embedded in wax it may be stored for treatment later.

After fixation and washing, the treatment prior to impregnation depends on the method used for dehydration. If ethyl alcohol is used for this purpose the tissue must afterwards be cleared. If dioxan (diethylene dioxide), or butyl alcohol, is used, a special clearing agent is unnecessary. The alternative methods are given below and are preceded by details of the additional technique not already described in Chapter II.

TECHNIQUE OF WAX-IMPREGNATION

The fixed, dehydrated, cleared tissue is gradually impregnated with molten paraffin wax. This is done by transferring pieces to small pannikins or wide and shallow specimen tubes (internal measurements: length, $3\frac{1}{2}$ " ; diameter, $1\frac{1}{8}$ "), filled with wax to a depth sufficient to cover the tissue and kept in an oven at constant temperature not more than 5° C. above the m.pt. of the wax used. Use paraffin wax of m.pt. 48° C.— 53° C.—*not higher*. If the wax used is of too high a melting point, the tissue may be cooked during the impregnation and difficulty may be found in getting a good ribbon of sections; over-hot paraffin causes shrinkage of the tissue.

Replacement of the clearing agent by the paraffin wax is easier if the cleared tissue is first transferred to a mixture of clearing agent and wax and then to pure molten wax. Care must be taken to avoid getting xylene or benzene in the wax in the final stage of impregnating.

If an oven is not available the pure wax for the final impregnation may be kept molten to a depth of about 1" in a small beaker by placing a small inverted gas burner about 2" above the wax. Or a small electric bowl fire may be inverted about 18" or 24" above the wax. These simple devices have one advantage in that objects on top of the semi-molten wax do not get over-heated. Further, several receptacles for wax can be used; and waxes of different melting points can be used by standing the receptacles on wooden blocks at different distances from the source of heat.

It is a matter of common sense to avoid using any naked light for heating vessels containing xylene, benzene, and other inflammable substances mixed with the wax. Instead, use carbon-filament electric bulbs, or a water-bath.

The wax used for the second and third impregnation baths should be used time after time. It improves by use and continued reheating. In fact, a good impregnating and embedding wax should have been in use twelve months or more.

Waddington and Kriebel* suggest adding 0.50% of

* *Nature*, 136, 685; Oct. 26, 1935.

petroleum ceresin to embedding wax to give a fine texture on cooling. Add the petroleum ceresin to a mixture of wax having a m.pt. slightly lower than that normally used.

When impregnating very fresh tissue, an acetone-paraffin wax mixture may be used instead of the benzene-wax or xylene-wax mixture.

For impregnating very small objects see the technique for their embedding, II (p. 92).

Further details of the mixtures and times involved in impregnating by different methods are given under Methods PW-1 (pp. 98-106); PW-2 (pp. 107-8); and PW-3 (pp. 109-10).

TECHNIQUE OF WAX-EMBEDDING

The wax-impregnated tissue is next embedded in a block of paraffin wax of size suitable for placing in the microtome. The block is made by pouring molten wax into a mould and, at the same time, the tissue is so placed and orientated in the mould as to enable it to be cut in the required plane.

It is first necessary to have some form of mould in which the tissue may be set and orientated. Three methods are indicated below.

(a) BRASS MOULD.

Cut two pieces of brass $\frac{1}{16}$ " thick, and $\frac{1}{2}$ " \times $1\frac{5}{8}$ ". Bend each piece into the shape of an L with the long side 1" and the short side $\frac{5}{8}$ " long. Stand on edge in the form of a box on a small sheet of glass. Warm before use.

(b) PAPER MOULD.

Make of stiff paper a box 1" \times $\frac{5}{8}$ " \times $\frac{1}{2}$ " deep according to directions * below. (See Fig. 17.)

1° Always fold the same way (letters inwards).

2° Fold aa' and bb'.

3° Fold cc' and dd'.

4° Fold AA' by applying Ac against Aa and pinching out AA'.

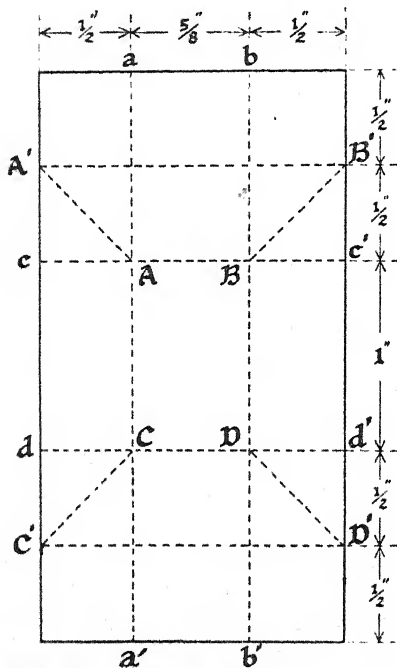
* Reproduced, with modification, by permission of Messrs. J. and A. Churchill, from Bolles Lee's *Microtometist's Vade-Mecum*.

5° Repeat for BB' , CC' , DD' .

6° Turn the "dog's ears" round against the ends of the box.

7° Turn down, outside, the projecting flaps that remain.

8° Smear the inside of the box with glycerine before use.



After Lee, *Microtometist's Vade-Mecum*, by permission.]

[J. G. Hawkes.

FIG. 17.—Lines of fold in paper for making embedding box.

(c) WATCH-GLASS.

Useful for small objects. Lightly smear the concavity of the glass with glycerine before use.

I. Normal Method of Wax-embedding.

1° Pour hot wax (m.pt. 48° – 53° C.) at not more than 55° C. into a warmed mould.

- 2° Warm some forceps and quickly place the impregnated tissue into the molten wax.
- 3° Orientate the tissue as desired with a warm needle.
- 4° Quickly add more molten wax to fill the mould, if necessary still holding the tissue in the desired position.
- 5° While the wax is still molten it is well to insert a slip of paper bearing a description of the tissue, so that the paper protrudes at one corner of the mould between mould and wax. This paper will stick to the wax on cooling. Unlabelled wax blocks are a source of confusion.
- 6° Hold the mould on the surface of some cold water, blow gently on the surface of the wax and, as soon as a pellicle has been formed, immerse the mould in the water. In immersing the mould hold it at an angle to the surface of the water to prevent too sudden pressure of water on the half-solidified wax. Quick cooling of the wax is essential to prevent crystallization and consequent difficulty in sectioning.
- 7° Remove the wax block from its mould. In this condition tissues may be preserved till they are required for sectioning.

II. Method for Wax-embedding Very Small Objects.

Place the object in a small piece of glass tubing. Tie a piece of silk over one end of the tube and invert the tube so that the object rests on the silk. Place the inverted tube in the wax to a suitable depth.

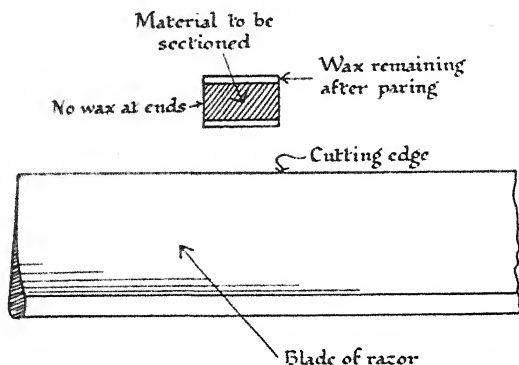
This method may be used for all processes from fixing to embedding.

TECHNIQUE OF SECTIONING WAX-EMBEDDED TISSUE

The block of wax-embedded tissue is now prepared for sectioning by either a hand or a mechanical microtome.

- 1° Remove the wax block from its mould and pare away the wax so that none is left at the sides of the tissue, very little

at the top and bottom, and so that the upper and lower surfaces of the block are parallel (Fig. 18).



[J. G. Hawkes.]

FIG. 18.—Relation of cutting blade and pared block.

Next, remove the corners of the block on its front face. This enables the wax ribbon to be divided easily when required.

Then (a) USING HAND MICROTOME.

2° Insert block in holder.

3° Hold the edge of the razor parallel with the long side of the block and, as in 2° (p. 23), cut sections of desired thickness, *but do not wet the razor blade.*

or (b) USING MECHANICAL MICROTOME.

2° Melt the wax on the chuck of the microtome with the heated blade of an old scalpel.

3° Press the block firmly on to the warmed chuck, arranging the long side of the block so that it will be parallel with the edge of the razor (Fig. 18).

4° Cool under tap.

5° Fix chuck on microtome and cut sections of desired thickness, usually 8μ – 12μ . Support the ribbon of sections on a finger of the free hand.

- 6° Throw the ribbon of sections on to the surface of a bowl of water just as hot as the hand will bear comfortably. (The hot water removes all wrinkles in the tissue and the wax.)
- 7° If desired, divide the ribbon (still floating on water) into portions by placing the slightly compressed arms of a pair of fine forceps one on each side of the partial junction in the ribbon (already made by removing the corners of the unsectioned block) and then allowing the forceps to open.

Now proceed to technique of attachment of impregnated sections to slide (p. 95).

Note: In using the mechanical microtome the sections should come off in a ribbon. If they do not:

- (i) The wax is probably too hard; warm it gently or coat the block with wax of m.pt. 40° C.
- (ii) The knife may be too cold; warm it gently.
- (iii) The knife may be greasy; wipe it with a cloth soaked in xylene.
- (iv) The upper and lower surfaces of the block may not have been pared parallel; remedy obvious.
- (v) The xylene used before the final impregnation may have been imperfectly removed; return the block for further impregnation in pure wax.

If the sections and tissue tear:

- (i) The razor may be blunt.
- (ii) The razor may be set at the incorrect angle (impossible on a good type of microtome).
- (iii) The tissue may not have been fixed properly.
- (iv) The fixative may not have been washed out.
- (v) Subsequent treatment, especially dehydration and impregnation, may have been imperfectly carried out.

Remedies for the above are obvious.

ATTACHMENT OF WAX-IMPREGNATED
SECTIONS TO SLIDE

For ease of manipulation after sectioning, the wax-impregnated sections are attached to a glass slide or, in some instances, to a glass cover-slip.

The adhesive used must be sufficiently strong to retain the section(s) attached to the slide, and must not dissolve off the slide during subsequent operations. On the other hand, it must in no way interfere with the final preparation. The substance in most common use is egg-albumen, either alone, or with glycerine; but human saliva is cheaper and very effective.

After the sections have been attached to the slide, the wax with which they were impregnated must be removed. This is done by dissolving it in xylene.

Technique of Attachment of Wax-impregnated Sections
to Slide.

- 1° Smear a *clean grease-free* slide very sparingly and evenly with egg-albumen or saliva.

(The edge of the palm of the hand drawn from end to end of the slide produces an even smear.)

- 2° Pass the prepared slide under the ribbon (or portion of ribbon, if it has been divided) and draw it on to the slide with the aid of surface tension. (See 6° and 7°, p. 94.)

- 3° Remove excess water by gently covering with filter paper.

- 4° Dry in an oven at 37° C. (Overnight).

[In urgent work :

- a° Leave in oven for as long as possible at 37° C.
(30 mins. minimum).

- b° Blot gently with a piece of filter paper saturated with alcohol (100%).

- c° Allow to dry. (5 mins.).]

SUBSEQUENT TREATMENT OF UNSTAINED
WAX-IMPREGNATED SECTIONS

As stated under the paragraph on staining, it is usually preferable to stain tissue in sections rather than in bulk. Con-

sequently the wax-impregnated sections attached to the slide are as yet unstained. Before they can be stained, the xylene used for dissolving out the wax must be removed. This is done by treating the sections with absolute alcohol.

Now the stain to be used may be dissolved in alcohol (usually 70% or 80%), or in water, so the sections attached to the slide must next be hydrated gradually, by treatment with alcohol (90%), then with the strength of alcohol in which the stain is dissolved, or, if an aqueous stain is to be used, with distilled water.

The reasons for gradual hydration will be appreciated by a reference to those advanced for gradual dehydration, but sections attached to slides are not harmed by passage from alcohol (90%) to water. Treatment after hydration will involve staining, differentiating, dehydrating, clearing, mounting, covering, labelling, and ringing.

Technique of Wax Removal and Hydration.

Note: Processes 1°-8°, inclusive, may be carried out by pouring the reagents *gently* on the slide and allowing them to drain off after an appropriate lapse of time; or by placing the slides back to back in flat-bottomed specimen tubes (internal measurements: length, $3\frac{1}{2}$ " ; diameter, $1\frac{1}{8}$ ") fitted with corks; or in slide holders (p. 283) placed in vessels of suitable size; or in more elaborate jars such as those supplied by Messrs. Flatters & Garnett, Manchester (Catalogue B, 6th edn., p. 74).

1° Warm slide *very* gently until the wax *just* melts.

2° Remove melted wax with xylene.

3° Remove xylene with alcohol (100%).

4° Hydrate thus[omit c°, d°, e°, if sections are attached to slide]:

a° Transfer to alcohol (100%). (1 min.).

b° Transfer to alcohol (90%). (1 min.).

[c° Transfer to alcohol (70%). (1 min.).]

If an alcoholic stain is to be used, proceed to 5°.

If an aqueous stain is to be used, proceed to d°.

[d° Transfer to alcohol (50%). (1 min.).]

[e° Transfer to alcohol (30%). (1 min.).]

f° Transfer to distilled water. (1 min.).

Subsequent Processes :

- 5° Stain.
- 6° Differentiate.
- 7° Dehydrate.
- 8° Clear.
- 9° Mount. Place balsam on section(s) on slide ; then cover.
- 10° Label.
- 11° Ring, if desired.

Details of the processes 5° to 8° inclusive will vary according to the staining and dehydrating methods used. A suggested technique is given in Method P-6, processes 22° to 32° (pp. 105, 106).

SUBSEQUENT TREATMENT OF STAINED WAX-IMPREGNATED SECTIONS

All that remains to be done with sections from bulk-stained material is to remove the wax from them, mount and cover.

Technique.

- 1° Remove wax from sections by *gently* pouring xylene on to the slide and allowing the solution to drain off.
- 2° Wash gently with fresh xylene.
- 3° Mount in Canada balsam by placing sufficient balsam on the slide to cover the section(s).
- 4° Cover.
- 5° Label.
- 6° Ring, if desired.

PERMANENT PREPARATIONS.

METHOD PW-1. Wax-embedding, Sectioning, and Triple-staining

Suggested material: Transverse sections of Earthworm
(*Lumbricus* or *Allolobophora* sp.).

Process	Comment
1° Kill in alcohol (30%).	
2° Fix in Zenker's solution. (Minimum 10 hours).	(p. 290.) Cut the dead worm into pieces no longer than 0.5 cm. and wash out any debris from intestine.
3° Wash in running water. (Minimum 24 hours).	(p. 14.)
4° Wash in alcohol (70%) to which iodine has been added. (Minimum 12 hours).	(p. 256.) To remove mercuric chloride. As colour disappears, add more iodine.

Subsequent treatment depends on the dehydrating agent used:

- If ethyl alcohol is used, proceed to Method A, p. 99;
- If butyl alcohol is used, proceed to Method B, p. 101;
- If cellosolve is used, proceed to Method C, p. 102;
- If dioxan is used, proceed to Method D, p. 103;
- If Solvax is used, proceed to Method E, p. 104.

METHOD PW-1

Continued from process 4° (p. 98)

*Method A. Using ethyl alcohol as dehydrating agent.**Process**Comment*

5° Dehydrate in alcohol (50%). (p. 34.)

(30 mins.).

6° Dehydrate in alcohol (70%).

(30 mins.).

Either,

7° Dehydrate in alcohol (90%).

(6 hours).

8° Dehydrate in alcohol (100%).

(6 hours).

9° Dehydrate in fresh alcohol

(100%). (6 hours).

10° Transfer to first clearing

liquid, viz. 25/75 clearing
agent/alcohol (100%).

(24 hours).

If clearing agent is cedar-wood oil, gently add the alcohol to the oil and gently add the tissue. As the oil penetrates, the tissue sinks.

11° Transfer to second clearing

liquid, viz. 50/50 clearing
agent/alcohol (100%).

(24 hours).

12° Transfer to third clearing

liquid, viz. 75/25 clearing
agent/alcohol (100%).

(24 hours).

Proceed after this to process
13° (p. 100).*Or, cheaper method (using either isopropyl alcohol, or acetone).*

7a° Dehydrate in industrial

methylated spirit (approx.
95%). (6 hours).

8a° Transfer either to isopropyl

alcohol; or, to acetone.

(6 hours).

<i>Process</i>	<i>Comment</i>
9a° Transfer either to fresh isopropyl; or, to fresh acetone. (6 hours).	
10a° Transfer either to 25/75 xylene/isopropyl alcohol; or to 25/75 xylene/acetone. (24 hours).	Xylene must be used as the clearing agent if this method is used.
11a° Transfer either to 50/50 xylene/isopropyl alcohol; or to 50/50 xylene/acetone. (24 hours).	
12a° Transfer either to 75/25 xylene/isopropyl alcohol; or to 75/25 xylene/acetone. (24 hours).	
13° Transfer to pure clearing agent. (24 hours).	See note (a), p. 106.
14° Transfer to first impregnating mixture,* viz. 50/50 clearing agent/molten paraffin wax. (1 hour).	Use wax of m.pt. 48° C.-53° C. and keep in an oven at constant temp. not more than 5° C. higher than the m.pt. of the wax used (p. 89).
15° Transfer to molten paraffin wax. (1 hour).	(p. 89.) ditto.

Now proceed to process 16° (p. 105).

* If clearing agent was cedar-wood oil, this mixture should consist of equal parts of cedar-wood oil, benzene, and molten paraffin wax.

METHOD PW-1

Continued from process 4° (p. 98)

*Method B. Using butyl alcohol as dehydrating agent.**

Butyl alcohol is miscible with water, with ethyl alcohol, and with paraffin wax.

*Process**Comment*

5° Transfer to this mixture :

Ethyl alcohol (100%)	50 c.c.	} \equiv 30% alcohol.	(1 hour).
Butyl alcohol	50 c.c.		
Distilled water	225 c.c.		

6° Transfer to this mixture :

Ethyl alcohol	50 c.c.	} \equiv 60% alcohol.	(1 hour).
Butyl alcohol	50 c.c.		
Distilled water	62.5 c.c.		

7° Transfer to this mixture :

Ethyl alcohol	50 c.c.	} \equiv 80% alcohol.	(2 hours).
Butyl alcohol	50 c.c.		
Distilled water	21.9 c.c.		

8° Transfer to this mixture :

Ethyl alcohol	50 c.c.	} \equiv 95% alcohol.	(2 hours).
Butyl alcohol	50 c.c.		
Distilled water	2.63 c.c.		

9° Transfer to normal butyl alcohol.

(2 hours).

10° Transfer to 50/50 butyl alcohol/paraffin wax.

This is best done by placing the tissue in fresh butyl alcohol and adding the wax in shreds, gradually, over a period of 3 hours, till the volume of liquid is about doubled. See comment about wax under process 14°, (p. 100).

Now proceed to process 16° (p. 105).

* Butyl alcohol is much cheaper than ethyl alcohol. The author is indebted to Mr. E. T. Saunders, M.A., of Bedford Modern School, for a note on this method.

METHOD PW-1

Continued from process 4° (p. 98)

Method C. Using cellosolve as dehydrating agent.

Cellosolve (ethylene glycol monoethyl ether) is miscible with water, with alcohol, and with xylene, but is not a solvent for paraffin wax.

N.B.—Tissue must *not* be in bulky pieces when cellosolve is used, or distortion and shrinkage will occur.

<i>Process</i>	<i>Comment</i>
5° Dehydrate in cellosolve. (30 mins.).	No gradation of strengths is necessary.
6° Transfer to xylene. (24 hours).	
7° Transfer to 50/50 xylene/ molten paraffin wax. (1 hour).	See comment about wax under Method A, process 14° (p. 100).
8° Transfer to molten paraffin wax. (1 hour).	

Now proceed to process 16° (p. 105).

METHOD PW-1

Continued from process 4° (p. 98)

Method D. Using dioxan as dehydrating agent.

Dioxan (diethylene dioxide) is miscible with water, with paraffin wax, and with xylene. Have ready a small wide-mouthed stoppered vessel in which have been placed a little anhydrous calcium chloride (covered with a piece of zinc gauze to prevent contact between tissue and calcium chloride) and the dioxan.

N.B. After fixation with liquids containing potassium dichromate (which is not soluble in dioxan) or mercuric chloride washing must be very thorough.

*Process**Comment*

- | | |
|--|---|
| 5° Transfer the washed tissue to the dioxan. (The dioxan can be used again for this purpose.) | (Small pieces—2-3 hours).
(Large pieces—12 hours). |
| 6° Transfer to 50/50 dioxan/paraffin wax. (Maintain at temperature just above that of the m.pt. of the wax.) | See comment about wax under process 14°, (p. 100).
(1 hour). |

Now proceed to process 16° (p. 105).

METHOD PW-1

Continued from process 4° (p. 98)

Method E. Using "Solvax" as dehydrating agent.

"Solvax" is a neutral colourless fluid; b.pt. about 80° C.; non-toxic; miscible with any strength of alcohol above 20%; dissolves paraffin wax.

<i>Process</i>	<i>Comment</i>
5° Dehydrate in alcohol (20%). (30 min.).	
6° Transfer to "Solvax." (2 hours).	
7° Transfer to mixture 50/50: "Solvax"/molten paraffin wax. (1 hour).	See comment about wax under Method A, process 14° (p. 100).

Now proceed to process 16° (p. 105).

METHOD PW-1 (continued)

<i>Process</i>	<i>Comment</i>
16° Transfer to fresh molten paraffin wax. (1 hour).	If benzene or xylene were used for clearing, take care not to let any get into this wax.
17° Embed. (Place the impregnated tissue in a mould of molten wax which is allowed to solidify and then trimmed to a convenient size.)	See p. 90 for detailed instructions. See note (a), p. 106.
18° Section.	See p. 92. By a hand-operated or by a mechanical microtome.
[19° Attach sections to slide.]	See p. 95 for detailed instructions. [Omit if loose sections desired. See note (b), p. 106.]
20° Remove wax from sections.	See p. 96.
21° Hydrate.	See p. 96. Allow water to penetrate sections gradually.
*22° Stain in Delafield's hæmatoxylin. (5 mins.).	Use diluted stain.
23° Wash in tap-water.	N.B. If sections are attached to slide, all processes from 20° to 29° inclusive may be carried out by placing the slide, with sections attached, in a capsule of suitable size.
24° Stain in Mallory's stain Solution A. (<i>Till red</i> ; 3-20 mins.).	
25° Wash in distilled water.	
26° Wash in Mallory's Solution B. (1 min.).	To fix the fuchsin.
27° Wash in distilled water.	For some results of staining, see "Mallory's Triple Stain" (p. 262).
28° Stain in Mallory's stain Solution C. (<i>Till blue</i> ; 5-20 mins.).	
29° Wash in distilled water.	Much of the "miliness" visible when an alcohol-treated preparation is placed in benzene or xylene is due to atmospheric moisture. Therefore, use drop-
30° Dehydrate in alcohol (95%) (industrial methylated spirits).	
31° Dehydrate in alcohol (100%).	

* Alternative methods of staining are given at II (i) (p. 123) and II (j) (p. 124).

<i>Process</i>	<i>Comment</i>
32° Clear in benzene-phenol. [See note (a), below.]	bottles for applying liquids to the slide in processes 30° to 32°, which would include sufficient dehydration even if from distilled water, <i>and which must be carried right through in a maximum of 30 seconds.</i> Alternately, cellosolve may be used for dehydration in 30° (1 min.) after which proceed to 32°.
33° Mount in Canada balsam.	Place the balsam on the sections if attached to slide.
34° Cover.	(p. 40.)
35° Label.	(p. 47.)
36° Ring if desired.	(p. 49.)

Notes.

- (a) Tissues may be kept in storage after process 13° of Method A (p. 100) ; after processes 17°, 18° (p. 105) ; or (by omission of process 19°) after process 32°, when sections would be stored in benzene.
- (b) Process 19° may be omitted and the remaining processes up to number 32° carried out with quantities of sections. This will obviate the use of the rather large quantities of reagents necessary to fill the capsules used for carrying the slides to which the sections have been attached.

PERMANENT PREPARATIONS.

METHOD PW-2. Wax-embedding, Sectioning,
Staining, and Counterstaining

[Special method for the preparation of quantities of sections of animal material (see note, p. 108) for class work.]

*Process**Comment*

- 1° Fix small pieces of fresh tissue in "corrosive-acetic."
(8 hours).
- 2° Wash in alcohol (96%) containing iodine. (12 hours).
- 3° Dehydrate in alcohol (100%); 2 changes.
(12 hours each).
- 4° Clear in benzene-phenol. (p. 39.)
(6 hours.)
- 5° Impregnate with 50/50 benzene/paraffin wax.
(1 hour).
- 6° Impregnate with pure paraffin wax. (1 hour).
- 7° Transfer to fresh paraffin wax. (1 hour).
- 8° Embed in paraffin wax. (p. 90.)
- 9° Cut sections at 8 μ . (p. 92.)
- 10° Flatten out and attach about 40 sections to a sheet of thin mica. See technique of attachment of sections to slide (p. 95).
- 11° Remove wax with xylene. (p. 96.)
- 12° Hydrate to alcohol (70%). (p. 96.)
- 13° Wash in alcohol (70%) containing iodine. (p. 256.)
- 14° Wash in distilled water.
- 15° Wash in sodium thiosulphate (hypo). To remove iodine (p. 284).
- 16° Wash in distilled water.
- 17° Stain in Delafield's hæmatoxylin. (5 mins.).
- 18° Wash in tap-water.
(10 mins.).

<i>Process</i>	<i>Comment</i>
19° Stain in eosin Y (aq.). ($\frac{1}{2}$ min.).	
20° Dehydrate either, in alcohol (70%), (90%), (96%), (100%). (Each poured on quickly); or, in cellosolve. (1 min.).	Dehydration must Y be done quickly because eosin, is rapidly washed out by alcohol (70%) and (90%). One dehydration in cellosolve is sufficient.
21° Clear in benzene-phenol.	
22° Coat with paraffin wax until required for class work.	
23° Cut mica into pieces of convenient size and shape for mounting under a cover-slip.	If desired, store at this stage.
24° Place mica pieces in xylene.	To remove wax prior to mounting.
25° Mount the piece of mica bearing the section, on a slide in Canada balsam.	
26° Cover.	
27° Label.	
28° Ring if desired.	

Notes :

1. This method may also be used for plant material, with suitable alterations. The following are suggested :
 - (a) In process 1°, fix in formalin-alcohol. (12 hours).
 - (b) In process 2°, wash in alcohol (70%) and (90%).
(6 hours each).
 - (c) Proceed with processes 3°-12° (inclusive).
 - (d) Omit processes 13°-16° (inclusive).
 - (e) For processes 17°-19° (inclusive), substitute any desired staining technique selected from those given on pp. 195-8. A particularly useful one is Method (c), p. 197, using safranin O and aniline blue W.S. (alc.).
 - (f) If all the processes of Staining Method (c) have been carried out (as shown on p. 197), dehydration in process 20° (above) will be in alcohol (100%).

- (g) Results of staining with safranin O and aniline blue W.S. (alc.): cell nuclei, cutin, lignin, suberin (\rightarrow red); cellulose and cytoplasm (\rightarrow blue).

2. Sections may be stored as follows:

- (a) In ribbons, between sheets of paper, after completion of process 9°. Not a very satisfactory method.
 (b) By transference to xylene, after process 9°, if it is *not* desired to proceed with mica mounts.
 (c) On mica, after processes 22° or 23°, as indicated above.

PERMANENT PREPARATIONS.

METHOD PW-3. Bulk Staining, Embedding, and Sectioning

N.B. This method involves staining the whole piece of tissue before impregnating and embedding. The method is useful if it is not desired to continue operations immediately after staining, because the tissue can be preserved in bulk at a suitable stage in dehydration. Although the method is quicker, staining is definitely less well controlled—there is liability to overstain the tissue at the edges—and at best it must be considered rather rough and ready and it is not recommended for most purposes. It happens to be rather suitable for the tissue suggested below.

Tissue suggested: Sexual organs of *Fucus*.

<i>Process</i>	<i>Comment</i>
1° Fix in osmium tetroxide (1%). (24 hours).	Beware irritant vapour from fixative.
2° Wash in running water. (12 hours).	
3° Harden in alcohol (70%). (24 hours).	
4° Stain in borax carmine. (24 hours).	(p. 230.)
5° Differentiate in acid alcohol. (15 mins.).	(p. 32.)
6° Dehydrate in alcohol (70%). (1 hour).	(p. 34.)
7° Dehydrate in alcohol (100%). (1 hour).	(p. 35.)

<i>Process</i>	<i>Comment</i>
8° Clear in 50/50 cedar-wood oil/alcohol (100%). (24 hours).	(p. 39.)
9° Clear in pure cedar-wood oil. (24 hours).	(p. 39.)
10° Impregnate in 25/25/25:: cedar-wood oil/benzene/paraffin wax. (1 hour).	(p. 89.)
11° Impregnate in molten paraffin wax. (1 hour).	(p. 89.)
12° Impregnate in fresh molten wax. (1 hour).	(p. 89.)
13° Embed in wax.	(p. 90.)
14° Section.	(p. 92.)
[15° Attach sections to slide.	(p. 95.)]
16° Remove wax from sections.	(p. 96.)
17° Mount in Canada balsam.	(p. 40.)

Note : Process 15° may be omitted and the sections treated in quantities in a watch-glass. After process 16° sections may be stored in xylene.

CHAPTER VI

METHODS FOR SPECIFIC MATERIAL

Notes

- (i) It is advisable to refer also to Chapter VII, in which will be found directions for making up stains, etc., with notes on their uses.
- (ii) Unless otherwise stated, the stains mentioned are suitable both for temporary and permanent preparations.
- (iii) Although ethyl alcohol is usually suggested as the dehydrating agent, it should be understood that any other suitable substance (pp. 33ff.) may be used, and the processes adjusted accordingly.
- (iv) If a specific method is not indicated, use one of the general histological methods: animal tissue (p. 117); plant tissue (p. 194).
- (v) For list of abbreviations, see page 2.

Methods

Acarine. To find if present in tracheæ of bee.

- 1° Lay the dead bee, ventral side uppermost, with head pointing forwards, under a hand-lens supported on a suitable stand.
- 2° Compress the head by means of forceps and insert a needle between the second and third pairs of legs.
- 3° Draw the head and front legs away from the thorax and expose the thoracic muscles and (sometimes) parts of the tracheal system liable to infestation.
- 4° Remove the chitinous ring adjacent to the exposed organs and the major portion of the trachea connected to the first pair of spiracles exposed. Or, the whole trachea may be removed.

- 5° The trachea may now be mounted for temporary examination. After some experience it will be possible to recognize the presence or absence of the parasite by the abnormal or normal appearance of the tracheæ as seen under the hand-lens.

Acidophil Granules.

St. Hæmatoxylin and eosin Y (→ red); iron hæmatoxylin and van Gieson's (→ yellow); Mallory's (→ yellow to orange).

Adrenal Body. See "Suprarenal Body" (p. 213).

Adrenalin. See "Suprarenal Body" (p. 213).

Agaricus.

(i) See "Fungi" (p. 155).

(ii) *Section gills* just before spores turn brownish-black.

Aleurone Grains (with crystalloids).

St. Eosin Y (alc.).

Algæ.

(i) *Fix.* Formalin-alcohol, formo-acetic-alcohol, Bouin's fluid, or chrome-osmium (1%).

(ii) *St.* Aniline blue W.S.; light green S.F. yellowish; magdala red; phloxine B (the two latter may be counterstained with aniline blue W.S.—See Chamberlain's method, below), Heidenhain's hæmatoxylin (mordant with liquor ferri).

(iii) *Chamberlain's method.**

1° Fix in chrom-acetic.

2° Wash in water.

3° Transfer to glycerine (10%).

4° Wash in alcohol (95%).

(Till all glycerine removed).

* Adapted from Chamberlain, *Methods in Plant Histology*, 1932, and *Stain Technology*, 2, 1927.

- 5° Stain in phloxine B. (12 hours).
 - 6° Decant stain and add alcohol (95%). (1 min.).
 - 7° Transfer to aniline blue W.S. (1 min.).
 - 8° Transfer to a clean dish and pour specially *weak* acid alcohol (p. 221) on to material. (3-4 secs.).
 - 9° Decant acid alcohol and wash in alcohol (95%). (3 changes).
 - 10° Dehydrate in alcohol (100%). (5 mins.).
 - 11° Examine microscopically. If the red colour is too pale—due to too prolonged treatment with acid alcohol—re-stain in phloxine B (30 mins.), re-wash in alcohol (95%) and dehydrate again. If the blue is too pale, re-stain in aniline blue W.S., re-wash in alcohol (95%), and dehydrate again.
 - 12° Transfer to Venetian turpentine (10%).
(*In a desiccator. 2 days—or until Venetian turpentine is of the viscosity of glycerine.*)
 - 13° Mount in Venetian turpentine.
 - 14° Ring.
- (Chromatophore → blue ; nucleoli → bright red ; pyrenoids → bright red.)
- (iv) *Methods for delicate specimens* : Useful for those algæ and fungi which curl and shrink in xylene as no separate fixation, clearing, or dehydration is required.
- (a) 1° Place lacto-phenol on slide.
 - 2° Add the object.
 - 3° Warm the slide till the lacto-phenol steams.
 - 4° *St.* Aniline blue W.S. (in lacto-phenol) and keep steaming. (30 secs.).
 - 5° Transfer to lacto-phenol on slide and remove excess stain by gentle warming. *Examine frequently under microscope.*
 - 6° *Mt.* Lacto-phenol.
 - 7° Ring with paraffin wax.
 - 8° Add coating of varnish paint to the wax.

- (b) 1° Fix in formo-acetic alcohol. (*Long immersion*).
 2° Wash in alcohol (50%) and then in distilled water.
 3° Stain in aniline blue W.S. aq.
 4° Dehydrate either
 (i) to alcohol (100%). (2 changes).
 or, cheaper method,
 (ii) to industrial spirit (approx. 95%) followed
 by two changes of isopropyl alcohol.
 5° Mount in Venetian turpentine (10%). (See
 p. 286.)
 6° Allow preparation to dry in a desiccator con-
 taining soda lime.
 7° Ring.
- (c) 1° Fix in formo-acetic-alcohol. (*Long immersion*).
 2° Wash in alcohol (50%) and then in distilled water.
 3° Stain in aniline blue W.S. aq.
 4° Wash in distilled water.
 5° Place either
 (α) in glycerine (10%);
 or,
 (β) in a solution consisting of
 Distilled water 5 grs.
 Industrial spirits 4 grs.
 Glycerine 1 gr.
 6° Allow the glycerine, or the solution, to concen-
 trate by evaporation at room temperature.
 (*Several days*).
 7° Mount in glycerine (p. 250), or in glycerine jelly
 (p. 250).
- (v) See also methods under "Chara" (p. 141), "Clado-
 phora" (p. 143), "Desmids" (p. 146), "Diatoms"
 (p. 146), "Euglena" (p. 152), "Fucus" (p. 155),
 "Hormidium" (p. 168), "Oedogonium" (p. 189),
 "Protococcus" (p. 202), "Spirogyra" (p. 210),
 "Tribonema" (p. 215), "Ulothrix" (p. 216),
 "Volvox" (p. 216) and Methods T-1 (p. 74) and
 T-2 (p. 75).

(vi) *To mount, and preserve green colour.*

See "Algæ," iii, p. 312, for preserving green colour.

Alimentary Canal.

(i) *Fix.* Do not fix small pieces of the wall but ligature a section, distend with the fixative (if possible by means of a glass syringe) and ligature a segment containing the fixative. Then cut ligatured segment out and place in fixative ["corrosive-formaldehyde" or formol-saline (5%)].

(ii) *St.* Delafield's hæmatoxylin and eosin Y; Mallory's.

(iii) *Clear.* Cedar-wood oil.

Amœba.

(i) See "Protozoa" (p. 202).

(ii) *Kill.*

(a) Steam, or dry heat, or osmium tetroxide vapour.

(b) Cultures may be killed and fixed with Bouin's fluid.

(iii) *St.* Aceto-carmine; Delafield's hæmatoxylin.

(Both by irrigation.) (p. 46.)

Amphibian Eggs. (i) *St. Living.* Nile blue sulphate.

(ii) See Rana (iii) (p. 205).

Amphioxus.

(i) *Fix.* Bouin's fluid.

(ii) *Before Dissection.* 1° Place in nitric acid (20%).

(2-3 days at room temperature).

2° Wash thoroughly.

(iii) *Nerve cord.* *St.* Methylene blue.

(iv) *Pharynx.* *St.* Borax carmine.

(v) *Epithelium.* *St.* Delafield's hæmatoxylin.

(vi) *Semi-whole mounts.*

1° After treatment as in (i) dissect away one whole side of the animal.

2° Dehydrate.

3° Clear in cedar-wood oil.

4° Either (a) Preserve in a tube of cedar-wood oil;
or (b) Mt. Canada balsam.

Amyloid (animal).

(Infiltration in morbid animal tissue.)

1° *St.* methyl violet 2B (1% aq. soln.).

(10 mins. at least.)

2° Rinse in water.

(Rapidly).

3° Differentiate in acetic acid (1%).

(Watch under microscope).

4° Wash in water.

5° Mount in Farrants' medium.

N.B. Methyl violet 2B is washed out by alcohol.

Amyloid substances (plant).

St. Hanstein's rosaniline violet (→ red).

Anemone (Sea).

(i) *Kill.* Hot Bouin's fluid.

(ii) *Fix.* Alcohol (70%).

Animals, Small.

To tease. Chromic acid (1%).

Animal Tissue.

(i) *Animal tissue should be fixed before staining.* Formol-saline (p. 248) is a good general fixative, but refer first to "Fixation" (p. 15) and to the specific tissue required.

(ii) *Preserve.* See "Preservation of Material" (p. 312).

(iii) *Small entire objects:*

Treat in quantities in the way recommended in Method P-3, p. 83.

St. (a) (*Temp.*). Methyl green.

(b) (*Perm.*). Borax carmine [see (v) I (a) (p. 117) and Method P-3 (p. 83)]; Mayer's alum carmine (not for marine specimens); Mayer's carmalum (not for marine specimens); Mayer's acid hæmalum (nuc.); picrocarmine [see (v) III (a), (p. 125)].

(iv) *Fresh tissue:*

St. Acid fuchsin (diluted 20 times with Ringer's soln.); safranin O (not in glycerine jelly mounts); Hofmann's W.S. violet; iron-aceto-carmine; safranin O (15 mins.) and picric-aniline blue W.S. (*time varies*). (The two latter stain the chromatin *); methyl green (temp.).

(v) *General Histology.*

N.B. Before starting any method, consult Chapter VII (p. 220) to find out all you can about the stain, its uses and how it is made up. Those methods most suitable for a beginner are indicated by a heavy line at the left-hand side. To avoid repetition, the final processes of dehydration, clearing, and mounting have not been listed unless special methods are desirable. In any case, details of the process will vary according to the dehydrating agent (ethyl alcohol, cellosolve, dioxan, or butyl alcohol) in use, and reference should be made to the appropriate section on dehydration (p. 34). Canada balsam is a satisfactory permanent mountant.

Full details of routine methods are given in Chapter IV, p. 74, and in Chapter V, p. 98.

I. *Single Staining.*

(a) BORAX CARMINE (p. 230).

An alcoholic nuclear stain for general use and especially for small, whole animals, (p. 83).

1° Dehydrate tissue up to and including alcohol (50%) (p. 34). If the tissue has been preserved in alcohol (70%) omit this stage and proceed direct to either (i) 2°, or (ii) 2°.

either (i) *Progressive Method:*

2° Transfer to stain diluted with alcohol (50%).

(Leave till stained sufficiently).

3° Wash in alcohol (70%).

* *Science Masters' Book*, Part II, Murray.

or (ii) *Retrogressive Method* :

2° Transfer to stock solution of stain.

(*Till overstained*).

3° Differentiate in acid alcohol (p. 221).

4° Wash in alcohol (70%).

(b) **MAYER'S ACID HÆMALUM** (p. 263).

An aqueous nuclear stain for sections, small whole animals and bulk tissue. Considered by some authorities to be superior to all other hæmatoxylin and hæmatins.

(i) *Progressive Method*.

1° Place in stock solution of Mayer's acid hæmalum diluted to 30% with *distilled* water.

2° Wash in *tap*-water.

(ii) *Retrogressive Method*.

1° Stain to excess.

(*About 5 hours for tissue in bulk*).

2° Differentiate in acid alcohol (p. 221).

3° Transfer to alcohol (50%).

($\frac{1}{2}$ hour for tissue in bulk).

4° Transfer to alcohol (30%).

($\frac{1}{2}$ hour for tissue in bulk).

5° Wash in tap- or alkaline water (p. 285).

(1 hour for tissue in bulk).

(c) **ANDERSON'S IRON HÆMATOXYLIN** (p. 226).

A useful nuclear stain for sections. This stain requires a mordant, therefore see page 27.

1° Stain in Anderson's iron hæmatoxylin.

(15 mins.).

2° Differentiate in acid alcohol (p. 221).

3° Rinse in distilled water.

Note : Hæmoglobin has a great affinity for iron hæmatoxylin. Do not mistake red blood corpuscles for nuclei.

(d) CHLORAZOL BLACK E. (p. 235).

An alcoholic stain useful for sections or bulk tissue.*
Stain sections. (15-30 mins.).

N.B. No mordanting or differentiating required.
If overstained, differentiate in terpineol or dilute
"Milton" (p. 266).

Nuclei (→ black); chromosomes (→ black); cytoplasm (→ grey in varying shades); chitin (→ green); secreted products (→ grey in varying shades); glycogen (→ pink or red).

II. Counterstaining.

(a) DELAFIELD'S HÆMATOXYLIN (p. 240) COUNTER-STAINED WITH EOSIN Y Aq. (p. 244).

An aqueous nuclear stain counterstained with an aqueous plasma stain, for general use and especially for sections.

1° Rinse the fixed tissue in *distilled water*.

2° Transfer to stock solution of Delafield's hæmatoxylin diluted 5 times with distilled water.
Do not overstain (excess hæmatoxylin is difficult to remove). (5-10 mins.).

3° Differentiate in acid alcohol (p. 221). (*Momentarily*).—(To remove the hæmatoxylin from the cytoplasm which, otherwise, would give a purplish-red colour when counterstained with eosin Y.)

4° Wash in *tap-water* or alkaline water (p. 285)
(to blue the hæmatoxylin). (5 mins.).

5° Counterstain with eosin Y aq. (30-60 secs.).

6° Rinse in *tap-water*.

7° Dehydrate alcohol (96%). (1 min.).

8° Dehydrate alcohol (100%). (1 min.).

* Recommended by H. Graham Cannon, *Nature*, 139, 3517, 549;
March 27, 1937.

* Acidophil granules	: blue.
Axis cylinders	: mauve.
Basophil granules	: blue.
Bone	: pink.
Calcifying matrix	: pale pink.
Cartilage matrix	: slate blue.
Collagen fibres	: pale pink.
Cytoplasm	: pink.
Dentine	: pink.
Elastic fibres	: bright pink.
Enamel	: pink.
Erythrocytes	: bright pink.
Keratin	: bright pink.
Lens fibres	: bright pink.
Muscle	: pink.
Nail	: bright pink.
Neuroglia fibres	: pink to mauve.
Nuclei	: blue.
Oxyntic cells	: bright pink.
Stratum lucidum	: bright pink.

- (b) ANDERSON'S IRON HÆMATOXYLIN (p. 226)
 COUNTERSTAINED WITH EOSIN Y Aq. (p. 244)
 1°-3° As I (c) 1°-3° (p. 118).
 4° Counterstain in eosin Y aq.
 5° Wash in distilled water.
 6° Dehydrate alcohol (96%). (1 min.).
 7° Dehydrate alcohol (100%). (1 min.).

- (c) ANDERSON'S IRON HÆMATOXYLIN (p. 226)
 COUNTERSTAINED WITH VAN GIESON'S (p. 248)
 1°-3° As I (c) 1°-3° (p. 118). Staining in hæmatoxylin must be prolonged [see (f) 1°, p. 122].
 4° Counterstain in van Gieson's. ($\frac{1}{2}$ -5 mins.).
 5° Wash quickly in distilled water.

* Reproduced by permission of Mr. Humphrey Milford from Hartridge and Haynes, *Histology for Medical Students*.

Note : Should the tissue be too deeply counterstained, excess red may be removed by water and excess yellow by alcohol.

* Acidophil granules	: yellow.
Axis cylinders	: grey.
Basophil granules	: black.
Bone	: black.
Calcifying matrix	: grey to black.
Cartilage matrix	: pale grey.
Collagen fibres	: red.
Cytoplasm	: yellow.
Dentine	: black.
Elastic fibres	: yellow.
Enamel	: grey to black.
Erythrocytes	: yellow.
Keratin	: black.
Lens fibres	: yellow.
Muscle	: yellow.
Nail	: black.
Neuroglia fibres	: yellow.
Nuclei	: black.
Oxyntic cells	: yellow.
Stratum lucidum	: yellow.

(d) BORAX CARMINE (p. 230) COUNTERSTAINED WITH
EOSIN Y ALC. (p. 244).

- 1° Overstain in borax carmine.
- 2° Wash in alcohol (70%).
- 3° Differentiate in acid alcohol (p. 221).
- 4° Wash in alcohol (70%).
- 5° Counterstain in eosin Y alc.
- 6° Dehydrate in alcohol (96%). (1 min.).
- 7° Dehydrate in alcohol (100%). (1 min.).

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METHODS FOR ANIMAL TISSUE

- (e) EOSIN Y AQ. (p. 244) COUNTERSTAINED WITH BORREL'S METHYLENE BLUE (p. 230).

1° Dilute some Borrel's methylene blue to 1 in 4 with distilled water.

2° In separate vessels warm some diluted methylene blue, and some eosin Y aq., both to 60° C.

3° Place the sections in a dish and pour the warm eosin Y on them. (2 mins.).

4° Transfer sections to another dish and pour on the warm methylene blue. (1 min.).

5° Differentiate in tap-water. (*Till excess stain removed*).

6° Differentiate in alcohol (70%) examining frequently under low power.

7° Transfer to alcohol (100%). (2 mins.).

- (f) DELAFIELD'S HÆMATOXYLIN (p. 240) COUNTERSTAINED WITH VAN GIESON'S (p. 248).

1° Place in Delafield's hæmatoxylin diluted to 1 in 5 with distilled water.

(*At least 15 mins. Otherwise the hæmatoxylin may be washed out by the picric acid contained in van Gieson's stain.*)

2° Rinse in tap-water or alkaline water (p. 285) (to blue the stain).

3° Counterstain in van Gieson's. (7-10 mins.).

4° Blot dry.

5° Transfer to alcohol (100%).

(*As short a time as possible*).

- (g) EHRlich'S HÆMATOXYLIN (p. 244) COUNTERSTAINED WITH EOSIN Y AQ. (p. 244).

1° Stain in stock solution of Ehrlich's hæmatoxylin. (5 mins.-24 hours).

2° Differentiate in acid alcohol (p. 221).

3° Wash in tap-water (p. 285).

(5 mins.-1 hour).

4° Counterstain in eosin Y aq. (1 min.).

- 5° Wash in water. (2 mins.).
- 6° Dehydrate in alcohol (96%). (1 min.).
- 7° Dehydrate in alcohol (100%). (1 min.).

(h) HEIDENHAIN'S HÆMATOXYLIN (p. 253) COUNTER-STAINED WITH EOSIN Y Aq. (p. 244).

Useful for cytological work.

- 1° Place in iron alum (2% aq.) (p. 256), or liquor ferri (p. 261).

[½-12 hours according to fixative used. Bouin, Carnoy, formaldehyde and "corrosive-formaldehyde" fixed material requires a short time (1 hour) in the mordant: algæ fixed in formo-acetic-alcohol should be mordanted in liquor ferri for about 4 hours].

- 2° Wash with distilled water.

- 3° Stain in Heidenhain's hæmatoxylin.

(For as long as the tissue was in the mordant).

- 4° Rinse in distilled water.

- 5° Differentiate in iron alum (2% aq.) or in liquor ferri (if this was used for mordanting) and then dip in distilled water. Repeat.

(Until, on watching under the lower power of the microscope, the colour is removed from almost all but the nuclei).

- 6° Immerse in running tap-water. (½ hour).

- 7° Counterstain in eosin Y aq. (2 mins.).

- 8° Wash in water.

- 9° Dehydrate alcohol (96%). (1 min.).

- 10° Dehydrate alcohol (100%). (1 min.).

(i) WEIGERT'S STAIN (p. 287) COUNTERSTAINED WITH VAN GIESON'S (p. 248).

A useful method for wax-embedded sections attached to slides.

- 1° Drain all water from loose sections, or xylene from sections attached to slide.

- 2° Rinse in alcohol (100%).
- 3° Rinse in alcohol (96%).
- 4° Stain in Weigert's. ($\frac{1}{2}$ –1 hour in a closed vessel).
This stain must *not* be poured on the slide.
- 5° Differentiate in acid alcohol (p. 221).
(1 min.).
- 6° Rinse in distilled water. (Quickly).
- 7° Counterstain in van Gieson's. ($\frac{1}{2}$ –5 mins.).
- 8° Wash quickly in distilled water.
- 9° Dehydrate. (Quickly).

Note : Should the tissue be too deeply counterstained, excess red may be removed by water and excess yellow by alcohol.

Collagen fibres (\rightarrow red, against a yellow ground); Elastic fibres (\rightarrow blue-black); Epithelia (\rightarrow yellow); Muscle (\rightarrow yellow).

(j) WEIGERT'S IRON HÆMATOXYLIN (p. 287) COUNTERSTAINED WITH VAN GIESON'S (p. 248).

A useful method for wax-embedded sections attached to slide.

- 1° Hydrate sections on slide (p. 96), alcohol (100%), (90%), distilled water. (1 min. each).
 - 2° St. Weigert's iron hæmatoxylin. (2 mins.).
 - 3° Wash off excess stain in distilled water.
 - 4° St. van Gieson's. ($\frac{1}{2}$ min.).
 - 5° Wash distilled water.
 - 6° Dehydrate alcohol (30%), (50%), (75%), (95%), (100%). (1 min. each).
- [Epithelia \rightarrow blue; connective tissue \rightarrow red; muscle \rightarrow yellow; nuclei \rightarrow blue.]

(k) EHRLICH'S HÆMATOXYLIN (p. 244) COUNTERSTAINED WITH EOSIN Y ALC. (p. 244).

A useful method for bulk staining.

- 1° Place in 1 part of stock, solution of Ehrlich's hæmatoxylin diluted with 2 parts of acetic acid (2%).

- 2° Transfer to fresh, diluted stain.
- 3° Rinse in *distilled* water.
- 4° Differentiate in acid alcohol (p. 221).
- 5° Wash in running tap or alkaline water (p. 285).
- 6° Dehydrate up to and including alcohol (90%).
- 7° Counterstain in eosin Y alc. (6 hours).
- 8° Wash in alcohol (96%).
- 9° Dehydrate in alcohol (100%).

III. Double Staining.

(a) Picro-Carmine (p. 272).

An aqueous nuclear double stain for general use and small whole animals.

Either (i) *Progressive Method* :

- 1° Place in stain diluted to 50% with distilled water. (*Leave till sufficiently stained*).
- 2° Wash in distilled water, and dehydrate.

Or (ii) *Retrogressive Method* :

- 1° Place in stock solution of stain. (*Till overstained*).
- 2° Dehydrate (p. 34) to alcohol (70%).
- 3° Differentiate in acid alcohol (p. 221).
- 4° Wash in alcohol (70%), and dehydrate.

(b) MANN'S DOUBLE STAIN (p. 263).

A good double stain for sections.

- 1° Fix tissue in Mann's fixative (p. 263).
- 2° Stain sections in Mann's stain. (24 hours).
- 3° Wash in water.
- 4° Dehydrate alcohol (30%), (50%), (70%), (90%), (100%). (2 mins. each).
- 5° Place in this solution :

Alcohol (100%)	50 c.c.
NaOH[1% in alcohol (100%)]	4 drops.

(*Till sections are red*).
- 6° Wash quickly in alcohol (100%).
- 7° Hydrate to water (p. 96).
(2 mins. to remove excess blue).

8° Transfer to water slightly acidified with acetic acid.

(Sections become blue and no more stain comes out).

9° Dehydrate.

Nuclei (→ blue); Connective tissue fibres (Collagen) (→ blue); Mucus-containing cells (→ blue); Basophil cell-granules (→ blue); Red blood corpuscles (→ red); Oxyphil cell-granules (→ red).

IV. Triple Staining.

(a) MALLORY'S TRIPLE STAIN (p. 262).

A good stain for routine work with sections.

1° Stain in solution A. (*Till red*; 3-20 mins.).

2° Wash in distilled water.

3° Wash in solution B. (*To fix the fuchsin*).

4° Wash in distilled water.

5° Stain in solution C. (*Till blue*; 5-20 mins.).

6° Wash in distilled water.

7° Dehydrate rapidly.

Brilmyer's Modification.

1° Stain Delafield's hæmatoxylin. (5 mins.).

2° Wash in tap-water.

3° Follow 1°-6° in (i) above.

*Acidophil granules	: yellow to orange.
Axis cylinders	: red.
Basophil granules	: black.
Bone	: blue.
Calcifying matrix	: blue.
Cartilage matrix	: blue.
Collagen (white) fibres	: blue.
Cytoplasm	: pink to red.
Dentine	: orange.
Elastic fibres	: red.
Enamel	: orange.

* Reproduced by permission of Mr. Humphrey Milford from Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

Erythrocytes	: yellow to orange.
Keratin	: bright red.
Lens fibres	: red.
Muscle	: red.
Myelin sheaths	: yellow to orange.
Nail	: bright red.
Neuroglia fibres	: red.
Nuclei	: red.
Oxyntic cells	: orange.
Stratum lucidum	: orange.

Annelids.

- (i) *Fix.* Chrom-acetic. (12 hours).
- (ii) See also "Hirudo" (p. 168), and "Lumbricus" (p. 174).

Anodon.

- (i) *To kill.*
 - (a) Plunge into boiling water. (1 min.).
 - (b) Put a wooden peg between the shells, and place in formaldehyde (10%).
- (ii) *Gill lamellæ.*
 - (a) The best sections are cut from embedded material, but quite good sections can, with care, be hand-cut from tissue held in a piece of carrot. Cut both horizontal—longitudinal, and vertical—transverse sections.
 - (b) *St.* Delafield's hæmatoxylin.
- (iii) *Glochidium.*
 - (a) Treat in quantities as suggested in Method P-3 (p. 83).
 - (b) *St.* Borax carmine.
 - (c) *Clear.* (i) Lactophenol,
or (ii) In a solution consisting of

Phenol	20 gr.
Glycerine	40 c.c.
Distilled water	40 c.c.

 Allow to evaporate at room temperature to half its original volume.

- (d) *Mount*. (i) After c(i) in lactophenol,
or (ii) after c(ii) in glycerine (p. 44).

Aphrodite.

- (i) *Kill*. Drown in water.
(ii) *Fix*. Formaldehyde (40%).

Aquatic and Marine Organisms (Small).

- (i) *To kill*.
(a) Allow the live specimen to expand in water, sprinkle menthol on the surface. Touch the specimen gently with a glass rod to test the degree of narcotization. The process is slow.
(b) Vapour of glacial acetic acid, or osmium tetroxide.
(c) *Specially useful for whelks and other molluscs*.
Carefully run "soda-water" from a syphon down the side of the vessel of water containing the animals.
(d) *Specially useful for ascidians, Balanus, sponges*.
Chloral hydrate (0.1%).
(e) *Celenterates and polyzoans*. See pp. 144 and 201.
(ii) *Small specimens*.
(a) *General treatment*.
Deal with quantities as suggested in Method P-3 (p. 83).
(b) *St*. Borax carmine.
(iii) *To entice from hiding*. Use Method (i) (c) above.
(iv) *St*. Borax carmine.

Arenicola.

- (i) *Kill*. Alcohol (70%).
(ii) *Fix*. Formaldehyde (10%).

Arthropods (Small terrestrial and aerial).

- (i) *To kill*. See "Killing Bottle" (p. 258).
(ii) *Permanent preparation*.
1° Kill and clear in Berlese's fluid (p. 229).
2° Mount in Berlese's fluid.

3° Dry off in oven.

4° Ring.

- (iii) See also "Crustacea" (p. 145) and "Insects" (p. 170).

Ascaris.

- (i) See "Nematodes" (p. 185).

- (ii) (a) *Section.* By embedding in wax. First roughen cuticle and make it wax-permeable by treating specimen with potassium hydroxide (5% aq.).
(Several hours).

- (b) *Stain sections.* Mallory's.

Aspidium.

To macerate the stele.*

Sodium hydroxide 2.5 gr.

Water 100 c.c.

Macerate for 1 month and wash in running water for not less than 24 hours.

Astacus.

- (i) *To kill.* Plunge into boiling water. (10 secs.).
(ii) *Fix.* Alcohol (70%).
(iii) *Small appendages.* First boil in potassium hydroxide (5% aq.). (Few mins.).
(iv) *Eye.* Use a fresh crayfish.
(a) *To see crystalline cones.* Mt. sections in potassium hydroxide (5% aq.).
(b) *To see rods.* Tease out with osmium tetroxide (1%).

Asterias.

- (i) *Kill.* Formaldehyde (5%).
(ii) *Fix.* Formaldehyde (10%).
(iii) *Sections.* (T.S. arm; T.S. disc; H.S. disc).

1° Decalcify in:

Alcohol (70%) 99 c.c.

Hydrochloric acid 1 c.c.

* Adapted from *Gerrard's Bulletin*, May 1939. T. Gerrard & Co.

2° Section at 10 μ .

3° *St.* Mallory's or borax carmine.

Atax ypsilophorus. (Mite on *Anodon* gills).

1° Alcohol (30%).

2° Berlese's fluid.

Bacteria.

(i) Grease-free slides and cover-slips are essential. See "Glassware—to clean" (p. 249).

(ii) A useful bacterium with which to acquire a technique is *Sarcina lutea* because it is quite large.

(iii) *St.* Loeffler's methylene blue; carbol-methylene blue; crystal violet; carbol-fuchsin (especially for cilia and flagella); basic fuchsin aq.; Bismarck brown Y.

(iv) *Temp.*

(a) 1° Place drop of infusion containing bacteria on a cover-slip and allow to dry in the air.

2° Invert the cover-slip in a drop of very dilute methylene blue on a slide.

3° Press gently with a clean filter paper.

(b) 1° As (iv) (a) 1°.

2° Place drop of very dilute methylene blue on the cover-slip.

3° Drain off excess stain on to filter paper.

4° Invert cover-slip in a drop of glycerine on a slide.

(c) *In zooglea stage.*

St. Iodine; to distinguish protoplasm (which stains) from gelatinous material (which does not stain).

(d) See Methods T-1 (p. 74), and T-2 (p. 75).

(v) *Perm.*

See note on mounting, under "Blood" (iii) (3) (b) (p. 134).

- (a) 1° With a sterilized platinum wire make a thin smear, on a slide, in a small drop of distilled water, spreading it to about 1 square inch.
- 2° Wave slide in the air to dry it.
- 3° Fix by passing, *when dry*, over a flame.
- 4° *St.* Aniline-crystal violet. (5 mins.).
- 5° Transfer to tap-water (to remove excess violet).
- 6° Transfer to Gram's iodine. (2 mins. until blackish).
- 7° Transfer to alcohol (90%). (Till no more colour is lost).
- 8° Transfer to Ziehl's carbolic-fuchsin (weak). ($\frac{1}{2}$ min.).
- 9° Transfer to tap-water. ($\frac{1}{4}$ min.).
- 10° Dry thoroughly over a flame.
- 11° *Mt.* Canada balsam.
- (b) 1° Smear a film of culture on slide with a sterilized needle.
- 2° Wave slide in air to dry.
- 3° Kill and fix by warming over a flame.
- 4° *St.* basic fuchsin aq. or other stain from selection above. (5 mins.).
Correctly stained slide should be almost colourless to naked eye.
- 5° Wash in distilled water.
- 6° Dry over a flame.
- 7° *Mt.* Canada balsam.
- (c) 1° Smear a film of culture on a cover-slip with a sterilized needle.
- 2°-6° As (v) (b) 2°-6°.
- 7° Place a drop of Canada balsam on a slide and invert the cover-slip on to it.

- (d) 1° Mix culture with Indian ink ("Pelican" brand).
- 2° Smear mixture on a slide, thinly.
- 3° Dry in the air.
- 4° *Mt.* Canada balsam.

[Bacteria show up "white."]

(vi) *Bacteria in tissues* :

St. Eosin Y(aq.) by one of the usual methods. This leaves the nucleoplasm of the bacteria unstained in contrast with the remaining tissue.

(vii) *Bacteria spores*.

- 1° Make a cover-slip smear as in previous methods.
- 2° Float cover-slip (inverted) on Ehrlich's aniline-water-fuchsin or Ziehl's carbolic-fuchsin (at 60° C.). (1 hour).
(This uniformly stains spores and protoplasm.)
- 3° Acid alcohol. (30 secs.).
- 4° Counterstain with methylene blue (aq. or alc.).
(Spores → red; rest of bacterial body → dark blue.)

Balanus.

- (i) *Kill and fix.* Formaldehyde (10%).
- (ii) See "Aquatic and Marine Organisms" (p. 128).

Barnacle. See "Balanus."

Basophil Granules.

St. Hæmatoxylin and eosin Y(→ blue); iron hæmatoxylin and van Gieson's (→ black); Mallory's (→ red).

Bast. See "Phloem" (p. 192).

Bean Cotyledon. See "Cotyledon" (p. 145).

Bean Root Tip. See "Mitotic Figures" (p. 177).

Beroë.*

- 1° Expand in sea-water.
- 2° Transfer to mercuric chloride (sat. soln. in sea-water). (*Till white*).
- 3° Decant. Add fresh sea-water. (*Several changes*).
- 4° Dehydrate to alcohol (70%), and store in this.

Bladder. Unstriated muscle fibres. See "Muscle (iv)" (p. 184).

Blood.

- (i) Grease-free slides and cover-slips are essential. [See "Glassware—to clean" (p. 249).]
- (ii) *Temp.*
 - 1° Prick the alcohol-sterilized lobe of the ear or ball of the finger with a sterilized needle. Do not squeeze the flesh unduly.
 - 2° Place *small* drop of blood on clean cover-slip. Do this by placing the cover-slip on the freshly exposed blood. Do not allow the slip to touch the skin.
 - 3° Dilute with sodium chloride solution (0.6% aq.).
 - 4° Invert the slip on a clean slide.
 - 5° Irrigate with acetic acid (1%). (Nuclei of erythrocytes in frog blood, and of leucocytes in frog and mammalian blood.)
 - 6° If the examination is prolonged, ring the slide with vaseline.
- (iii) *Perm.*
 - (1) *Fix.*
 - (a) *Erythrocytes and white corpuscles.*
"Corrosive-formaldehyde."
 - (b) *Leucocytes of invertebrate blood.*
Bouin's tinged with iodine.
 - (2) *St.*
 - (a) Eosin Y; acid fuchsin; Giemsa's; Leishman's; methylene blue; methyl violet (1%); Wright's.

* After Bolles-Lee, *Microtomet's Vade-Mecum*, Churchill.

(b) *Erythrocytes.*

Hæmatoxylin and eosin Y (\rightarrow bright pink); iron hæmatoxylin and van Gieson's (\rightarrow yellow); Mallory's (\rightarrow yellow to orange); hæmatoxylin and picric acid.

(3) *Mt.*

(a) Canada balsam.

(b) "Durofix" is particularly useful when it is desired to use a $\frac{1}{12}$ " objective, as, for example, in work with trypanosomes. Preparations stained with Giemsa's stain keep their colour better than when mounted in balsam.

Method: Do not use a cover-slip. Make the smear on a slide [as in iv (d), p. 135] and, after preparing it by any of the methods given in iv (below), make a thick line of "Durofix" at one end of the slide and distribute it evenly by a single movement of one finger along the slide.

(iv) *Useful methods.*

- (a) 1° Place a small drop of blood in the centre of a cover-slip. [See (ii) 1° and 2°, p. 133.]
- 2° Cover with another slip (2 secs.) and then draw the slips apart, laterally.
- 3° Wave in the air to dry.
- 4° Dip cover-slip in satd. soln. of eosin Y in alcohol (75%). (1 min.).
- 5° Alcohol (75%). (5 secs.).
- 6° Alcohol (75%) fresh. (5 secs.).
- 7° Satd. soln. of methylene blue in alcohol (75%). (5 secs.).
- 8° Alcohol (75%). (Momentarily).
- 9° Press slip *slightly* on pad of filter paper.
- 10° Wave in air to dry.
- 11° Invert slip in drop of Canada balsam on slide.

- (b) 1°-3° As (iv) (a) 1°-3° (p. 134).
4° Fix in formaldehyde vapour. (1 min.).
5° Fix in alcohol (100%). (1 min.).
6° Stain according to method II (a), p. 119, proceeding from process 2° onwards.
7° Clear in xylene.
8° *Mt.* in Canada balsam.

- (c) 1°-3° As (iv) (a) 1°-3° (p. 134).
4° Leave in 15 drops Giemsa's, Leishman's, or Wright's stain. (The methyl alcohol used in the stain fixes the tissue.) (30 secs.).
5° Add 15 drops distilled water. (6 mins.).
6° Wash rapidly in a stream of water till smear is red in colour. (*Usually* $\frac{1}{2}$ min.) Be very careful not to wash too long or nuclear stain is lost.
7°-9° As (iv) (a) 9°-11° (p. 134).

*Basophil granules (mast cells) (\rightarrow purple); Blood platelets (Giemsa's \rightarrow purple; Leishman's \rightarrow pink-purple); Eosinophil granules (\rightarrow red); Erythrocytes (Giemsa's \rightarrow bluish; Leishman's \rightarrow orange-pink); Leucocyte nuclei (Giemsa's \rightarrow purple; Leishman's \rightarrow blue-purple); Lymphocyte cytoplasm (Giemsa's \rightarrow dark blue; Leishman's \rightarrow bluish); Neutrophil (polymorph) granules (Giemsa's \rightarrow reddish violet; Leishman's \rightarrow purple); Parasites (\rightarrow blue with bright red chromatin).

- (d) 1° Place drop of blood on slide and smear by drawing the edge of the end of another slide over it.
2° Dry over an electric lamp.
3° *St.* Giemsa's, Leishman's, or Wright's stain. (5 mins.).
4° Wash in water.
5° Dry over an electric lamp.

* After Carleton, *Histological Technique*, and Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

6° *Mt.* Canada balsam or "Durofix" [see (iii) (3) (b), p. 134.]

For results see (c), p. 135.

(e) 1°-3° As (iv) (a) 1°-3°, p. 134.

4° Place about 10 drops of Wright's stain (q.v.) on the cover-slip.

(1-3 mins. *Time will depend on the stain.*)

5° Add about 10 drops of distilled water, drop by drop. (*Twice as long as staining occupied.*)

6° Pour off the stain.

7° Wash with distilled water.

(*Until thin areas of film are pink.*)

8° Dry on filter paper.

9° *Mt.* Canada balsam.

For results see (c), p. 135.

(v) *Leuco-malachite test for blood stains.*

1° Remove a small quantity of the (blood?) stain to a filter paper with a *clean* knife.

2° Add *one drop* of the leuco-malachite reagent (p. 260).

If the stain is blood, a green stain (*not* small, isolated, green points) will appear after not more than ten *seconds*. After the lapse of not more than one *minute* the green colour will change to a dark, greenish blue.

(vi) *Precipitin test for blood origin (human or non-human).*

1° Dissolve some of the blood stain in saline solution, for a few hours.

2° Filter.

3° Allow about $\frac{1}{2}$ " column of the liquid to be drawn up into a *clean* capillary tube. [See "Glassware—to clean" (iv) (p. 249).]

4° Allow a similar volume of "rabbit anti-human serum" to be drawn into the other end of the tube, so that the two liquids mix.

If the blood is human blood, a white precipitate or "colour" slowly forms.

- 5° It is essential to carry out the following controls :
- a° Will the given "rabbit anti-human serum" precipitate from *known* human blood ?
 - b° Will the saline extract of the blood stain fail to give a reaction with normal rabbit serum ?
 - c° Will both the "rabbit anti-human serum" and the saline extract of the blood stain fail to react with the saline alone ?

Blood Parasites. See "Blood" (iv) (c), (d) and (e), pp. 135, 136.

Blood Stains. See "Blood" (v) and (vi) (p. 136).

Blood Vessels.

- (i) *Fix.* Formol-saline.

Note : Small vessels may be stretched on filter paper laid face down in fixative.

- (ii) *St.* Hæmatoxylin and van Gieson's.

Bone.

(i)

- 1° Fix thin slices in formol-saline.
- 2° Decalcify (see p. 239).
- 3° Wash out decalcifying medium.
- 4° Usual processes leading to embedding and sectioning.
- 5° *St.* Hæmatoxylin and eosin Y (→ pink); iron-hæmatoxylin and van Gieson's (→ black); Mallory's (→ blue).

- (ii) Mount unstained sections in "Euparal."

- (iii) *Dawson's method for whole mounts.** (*To stain the skeleton but not the flesh.*)

- 1° Fix in alcohol (95%).
(*Several weeks : until complete penetration has ensued.*)

- 2° Transfer to acetone.
(*Time will depend on size of object—not less than 14 days.*)

* With acknowledgements to R. W. Bateman.

- 3° Transfer to alcohol (95%).
(*Time will depend on size of object—not less than 14 days*).
- 4° Macerate in potassium hydroxide (2%).
(*Until bones show through flesh clearly*).
- 5° Stain with alizarin red S.
(*Until bones thoroughly stained*).
- 6° Clear in Moll's solution.
(*Until flesh transparent*).
- 7° Transfer to glycerine (50% aq.).
- 8° Transfer to glycerine (pure).
(*2 washings.*) (= preserving agent).

Bone Marrow (from rib).

- 1° Make a smear of bone marrow (on a slide or cover-slip).
by either,
 - (a) squeezing a piece of rib with the forceps until the marrow exudes,
 - or,
 - (b) splitting the rib and smearing the slide (or cover-slip) on the marrow *in one direction only*.
- 2° Wave in the air to dry.
- 3° Proceed as for blood films (iv) (p. 134).

Brain.

- (i) *Fix.* Formaldehyde (10%).
- (ii) *Harden.* 1° Formalin-dichromate. (2–3 weeks).
2° Wash in running water. (12 hours).

Bryozoa. See "Polyzoa" (p. 201).

Bundle Ends. To distinguish venation.

(After Ashby, *School Science Review*, vol. XV, 1934. Murray.)

- 1° Place material in eau de Javelle (fresh).
(24–28 hours).

- 2° Wash running water (in a beaker covered with muslin).
(24 hours).
- 3° *St.* Ammoniacal fuchsin.
- 4° Wash in methylated spirit.
- 5° Dehydrate.
- 6° Clear.
- 7° *Mt.* Canada balsam.
(Lignified tissue → red; unlignified tissue → clear,
colourless.)

Calcifying Matrix.

St. Hæmatoxylin and eosin Y (→ pale pink); iron hæmatoxylin and van Gieson's (→ grey to black); Mallory's (→ blue).

Capillaries. To see in the finger.

Place a drop of cedar-wood oil on the skin above the nail-bed of the 4th digit. Place the finger on the microscope stage and view under low power by direct light in bright daylight.

Carrot. See Method T-3 (p. 76).

Cartilage.

- (i) *Fix.* Weak formol-saline.
- (ii) *St.* *Weigert's stain and van Gieson's (collagen fibres → red, elastic fibres → blue-black, matrix → grey black); *Mallory's (collagen fibres → blue, elastic fibres → red, matrix → blue); *hæmatoxylin and eosin Y (collagen fibres → pale pink, elastic fibres → bright pink, matrix → slate blue); methyl green (nuclei and intercellular matrix → green); orcein and van Gieson's [elastic fibres (nuclei → blue), (cytoplasm → brownish-pink); connective tissue → red].

* Adapted, by permission, from Hartridge and Haynes's *Histology for Medical Students*.

(iii) *van Wijhe's method for whole mounts.** (To stain the skeleton but not the flesh.)

1° Fix in alcohol (70%). (Until complete penetration).

2° Stain in toluidine blue O, or thionin, or new methylene blue N. (3 weeks).

3° Transfer to acid alcohol.

(Till no more stain removed).

4° Dehydrate in alcohol (70%).

5° Dehydrate in alcohol (90%).

6° Dehydrate in alcohol (100%).

7° Transfer to benzene.

8° Clear in oil of wintergreen. } (Several washes).
Spalteholtz clearing method.

(= preserving agent).

Times will vary with size of object but in any case will probably be not less than 14 days.

Cartilage → blue; other tissues unstained. It is possible to clear and preserve in cedar-wood oil. Small pieces may be mounted in Canada balsam.

Cell Outlines. (To demonstrate cement substance.)
See "Epithelia" (vi) (p. 151).

Cellulose.

(i) Well seen in date stone. Soak for 24 hours in water and scrape off endosperm with heel of razor.

(ii) *St.* (a) *Temp.*

Schulze's soln. (specific stain) (→ blue violet) [reaction quicker by few minutes' treatment with KOH (5%) to remove fats beforehand]; corallin; iodine (→ brown) [+ sulphuric acid (approx. 40%) → blue].

(b) *Perm.*

Delafield's hæmatoxylin (→ purple); aniline blue W.S.; light green S.F. yellowish; (all counterstained with safranin O); acid fuchsin; Mayer's carmalum; (both

* With acknowledgements to R. W. Bateman.

counterstained with iodine green); safranin O (counterstain with cyanin); Hanstein's rosaniline-violet (\rightarrow pale violet); xylene-eosin Y (\rightarrow red); xylene-erythrosin bluish (\rightarrow red).

Cell Wall Stratification.

St. Hanstein's rosaniline violet (\rightarrow pale violet).

Cell Walls (Unlignified).

St. Delafield's hæmatoxylin (counterstain with aq. eosin Y); Heidenhain's hæmatoxylin (\rightarrow light blue).
See also "Cellulose" (p. 140).

Cement Substance (in Cell Outlines). See "Epithelia" (vi) (p. 151).

Cestodes. See "Tænia" (p. 213).

Chara.

- (i) *St.* Iodine and basic fuchsin.
- (ii) See also "Algæ" (p. 112) and "Cladophora" (p. 143).

Chick Embryo.

For the beginner it is useful to commence with an egg incubated for 60 hours.

(a) *Entire embryo:*

- 1° Place the egg in a dish of warm sodium chloride soln. (0.6% aq.).
- 2° Chip the shell away.
- 3° Cut round the embryo with scissors.
- 4° Slide the embryo off into the saline.
- 5° Wash off the yolk.
- 6° Shake off the vitelline membrane.
- 7° Transfer embryo to a cover-slip (this prevents curling).

8° Fix in Bouin's fluid diluted with 50% of its own volume of distilled water.

(Small—1 hour. Large—12 hours).

9° Wash in alcohol (50%), (70%). (Twice in each).

10° *St.* Borax carmine. (3-4 hours).

11° Dehydrate from alcohol (70%).

12° Clear in clove oil. (Overnight).

13° Pass quickly through xylene.

14° *Mt.* Canada balsam. (See p. 41 for mounting thick specimens.)

(b) *Sections :*

1° As (a) up to and including 13°.

2° Remove from cover-slip and transfer to bath of pure wax at 50° C. (30 mins.).

3° Embed in wax (see p. 90).

4° Cut sections 10 μ -12 μ thick (see p. 93).

5° Attach sections to slide (see p. 95).

6° Remove wax from sections (see p. 96).

7° *Mt.* Canada balsam.

Chitin.

(i) *St.* Picric acid, aq. or alc. (\rightarrow yellow) ; picro-clove oil.

(ii) *To identify in fungus.**

1° Cut fungus into small pieces.

2° Boil with dilute potassium hydroxide.

3° Boil with dilute sulphuric acid.

4° Transfer to alcohol (70%).

5° Transfer to ether.

6° Decant ether, and dry the white residue : it is chitin.

Chlorine Ions.

St. Silver nitrate.

Chloroplasts.

St. Hanstein's rosaniline violet.

* After Strasburger, *Text-Book of Practical Botany*, Allen and Unwin.

Chromatin.(i) *Animal.*(a) *General.*

St. Delafield's hæmatoxylin (→ purple).

(b) *In fresh tissue.*†

St. Safranin O and picric-aniline blue W.S.;
iron-aceto-carmin.

(c) *In blood parasites.*

St. Leishman's (→ ruby red).

(ii) *Plant.*

St. Safranin O (→ red).

Chromatophores.

St. Acid fuchsin.

Chromosomes. See "Mitotic Figures" (p. 177).**Cilia.**

St. Carbol fuchsin; iodine.

Cladophora.

- (a) *Fix.* Chromic acid (1%); osmium tetroxide (1%);
picric acid (sat. aq.); chrom-acetic; (*All for 24*
hours). Chrom-osmium-acetic ($\frac{1}{2}$ hour).

Wash. Running water. (12 hours).

- (b) *St.* Alum-carmin; ammonia-hæmatin,* thus:

1° Use picric-fixed tissue.

2° Transfer to several changes of boiled water.
(24 hours).

3° Place a few crystals of hæmatoxylin in a little
distilled water in a beaker.

4° Blow ammonia gas into the liquid.

5° Stain in the solution (much diluted with distilled
water). (3 hours).

6° Wash in distilled water.

Cockroach. See "Periplaneta" (p. 191).

† *Science Masters' Book*, Part II, Murray.

* After Strasburger, *Text-Book of Practical Botany*, Allen and Unwin.

Cœlenterates.(i) *To kill.*

Allow the live specimen to expand in water. Add crystals of magnesium sulphate from time to time. Do not allow the crystals to drop on the specimen. Test state of narcotization with a glass rod. The process is slow. See also "Hydra" (p. 168).

(ii) *General treatment of small specimens.*

Deal with quantities in the way recommended in Method P-3 (p. 83).

(iii) *Mounting small specimens.*(a) *Specimens with tentacles* (e.g. *Hydractinia*; *Tubularia*; etc.).

1° Place drop of Canada balsam on the slide.

2° Push the specimen along the slide, tentacles first, into the balsam to ensure displaying to full advantage.

(b) *Medusæ.*

Do not use pressure on the cover-slip.

- (iv) See also "Hydra" (p. 168), "Obelia" (p. 188), "Beroë" (p. 133), "Pleurobrachia" (p. 200).

Collagen (White) Fibres. See "Cartilage" (p. 139) and "Connective Tissue" (below).

Connective Tissue.

- (i) *Fix.* Formol-saline (4%); Bouin's; "corrosive-formaldehyde."

(ii) *St.* (a) *White fibres (collagen).*

Eosin Y aq.; van Gieson's (→ red); acetic acid (1%) (white fibres disappear, yellow fibres defined); Mann's (→ blue). See "Animal Tissue" (p. 116), and "Cartilage" (p. 139).

(b) *Yellow elastic fibres (elastin).*

Weigert's stain (→ blue-black); Mallory's (→ blue).

Cork. See "Suberin" (p. 212).

Cotyledon.

Sections.

- (a) *St.* Iodine (3 drops to small watch-glass of water).
- (b) *Mt.* Glycerine jelly.

Crab.

Kill and fix. Formaldehyde (10%).

Crustacea (Small).

- (i) See also "Animal Tissue" (iii) (a), p. 125.

Carry out the processes with a quantity of specimens in the way recommended in Method P-3 (p. 83).

- (ii) 1° Kill and fix in Bouin's fluid, or "corrosive-acetic," or alcohol (30%).
- 2° Wash in alcohol (50%) 3-4 times [no washing if alcohol (30%) is used to fix].
- 3° Dehydrate to alcohol (70%).
- 4° *St.* Borax carmine.
- 5° Wash alcohol (70%).
- 6° Dehydrate from alcohol (70%).
- 7° Clear in clove oil.
- 8° *St.* Picro-clove oil (chitin → yellow or brown).
- 9° Wash in clove oil.
- 10° Pass through xylene.
- 11° *Mt.* Canada balsam.

Note: 4°-6° may be omitted and dehydration in 3° will be carried to alcohol (100%).

Ctenophores. See "Beroë" (p. 133).

Cutin.

St. (a) *Temp.*

Schulze's soln. (→ yellow brown).

(b) *Perm.*

Alkannin (→ red) (slow); safranin O (→ pink); Sudan IV (5% alc.); Sudan III (slow).

Cyclops. See "Crustacea (Small)" (p. 145) and Method P-3 (p. 83).

Cytology.

- (i) *St.* Heidenhain's hæmatoxylin (cell walls → blue; nuclei → dark blue; mitotic figures → black); Newton's crystal violet (mitotic figures → black).
- (ii) See also "Mitotic Figures" (p. 177).

Cytoplasm.

St. Hæmatoxylin and eosin Y (→ pink); erythrosin bluish; orange fuchsin; acid fuchsin; iron hæmatoxylin and van Gieson's (→ yellow); Mallory's (→ pink to red); Leishman's (→ bluish).

Daphnia. See "Crustacea (Small)" (p. 145) and Method P-3 (p. 83).

Dentine. See "Teeth" (p. 214).

Dermis. See "Connective Tissue" (p. 144).

Desmids.

- (i) *St.* Leishman's.
- (ii) To mount and preserve green colour. See "Algæ" (iv) (p. 312).

Diatoms—To Mount.

- (i) *A° Prepare some grease-free slides and cover-slips either by the method given under "Glassware, to clean" (p. 249), or, clean the slides by rubbing them with a green ink-eraser and water until the water remains evenly all over the glass when the slide is held on edge to drain it off.

B° Have ready

- (a) a fountain-pen filler with rubber bulb;

* Adapted, by permission, from Messrs. Watson's *Microscope Record*.

- (b) a bristle, as thick as a fine sewing needle and such as may be obtained from a good-quality hairbrush. The bristle should be sealed, by means of sealing-wax, into a small hole drilled in the end of a piece of brass wire, No. 12 B.W.G., heated over a non-luminous flame. For convenience of revolving, the other end of the wire should be twisted into a knob ;
- (c) Some adhesive, e.g. :
 - (i) glycerine-egg albumen ;
 - or (ii) glycerine-dextrin (best for large slides and does not dry) ;
 - or (iii) for delicate specimens, either (i), or (ii), diluted with an equal volume of distilled water.

C° *Method.*

- 1° Place the material containing the diatoms in a vessel of distilled water.
- 2° By means of a fountain-pen filler collect water containing diatoms and spread them over a slide cleaned as above.
- 3° Remove excess water with filler held at one corner of the tilted slide.
- 4° Dry off remaining water at room temperature.
- 5° Prepare a slide with adhesive by rubbing a drop of adhesive over the slide with the tip of the clean finger. Wipe off repeatedly till only a trace of adhesive remains.
- 6° Place the spread slide of diatoms on the microscope stage, focus the required diatom and pick it up on the tip of the bristle. If it does not instantly adhere, dip the tip of the bristle in a little adhesive.
- 7° Inspect the diatom for cleanliness. If it is not clean, breathe on a clean slide and rub the diatom (on the end of the bristle) in the moisture from the breath. If this is inadequate, use a drop of distilled water.
- 8° Have the slide covered with the film of adhesive ready on the stage of another microscope. Lower the

diatom on to the adhesive. Saucer-shaped diatoms should be mounted with the rim on the slide, i.e. so that the convex side is towards the objective.

- 9° Heat the slide for 5 mins. (fairly high temp.) to drive off the glycerine and harden the dextrin or egg-albumen.
- 10° Cool the slide. Clear the diatoms by adding a drop of benzene or xylene.
- 11° Apply a drop of Canada balsam [or "Hyrax" or "Sirax," see (ii) 8°, below] to the mounted diatom and cover with a clean slip. If necessary, raise the cover-slip by a suitable device (see "Mounting") (p. 41).
- 12° Heat quickly to drive off benzene (or xylene).
- 13° Ring if desired.

Note: If it is found that the hand trembles too much to mount accurately, the mounted bristle may be clamped to the microscope tube and lowered up and down by means of the focussing rack. The diatom is then positioned by lateral movements of the slide made by hand.

(ii) *Bause's Method.*

- 1° Fix in formo-acetic alcohol. (A long time).
- 2° Wash in water.
- 3° Stain in Heidenhain's hæmatoxylin.
- 4° Transfer to glycerine (10%) and allow this solution to concentrate in air at room temperature.
- 5° Transfer to pure glycerine.
- 6° Transfer to Venetian turpentine. (See p. 286.)
- 7° Transfer to xylene (to remove Venetian turpentine).
- 8° Mount in Canada balsam (or in "Hyrax" or in "Sirax," both of which, being of greater refractive index, give greater transparency).
- 9° Cover.
- 10° Ring.

Distomum. See "Fasciola" (p. 152).

Doris.

- (i) *Kill.* Drown in water.
- (ii) *Fix.* Alcohol (70%).

Ear.

- (i) See "Animal Tissue" (p. 116).
- (ii) *Elastic fibres.* *St.* Weigert's stain.

Earthworm. See "Lumbricus" (p. 174).

Echinus.

- (i) *Kill and fix.* Formaldehyde (10%).
- (ii) *Ova.* Treat in quantities in a tube as recommended in Method P-3 (p. 86). Processes 3° and 4° (below) may be omitted if desired.
 - 1° Fix in formaldehyde (3%). (2 mins.).
 - 2° Wash in alcohol (50%). (2 mins.).
 - 3° Stain in borax-carmin. (2 mins.).
 - 4° Differentiate in acid alcohol.
 - 5° Dehydrate in alcohol (70%).
 - 6° Dehydrate in alcohol (90%). (3 mins.).
 - 7° Dehydrate in alcohol (95%). (3 mins.).
 - 8° Transfer to mixture.
 - Alcohol (100%) 3 vols.
 - "Euparal Essence" 1 vol. (*Up to 15 mins.*).
 - 9° Transfer to mixture
 - Alcohol (100%) 2 vols.
 - "Euparal Essence" 2 vols. (*Up to 15 mins.*).
 - 10° Transfer to mixture
 - Alcohol (100%) 1 vol.
 - "Euparal Essence" 3 vols. (*Up to 15 mins.*).
 - 11° Transfer to "Euparal Essence." (*Up to 15 mins.*).
 - 12° Mount in "Euparal."

Elastic Fibres.

- (i) See "Cartilage" (p. 139).
- (ii) Acetic acid (1%) defines the fibres; orcein.

Embryo Chick. See "Chick Embryo" (p. 141).

Embryo Plants.—To see.

Soak some very young, *developing* seeds of *Capsella bursa-pastoris* (Shepherd's Purse) in dilute potash soln. Mount in water. Press gently under cover-slip and examine crushed seeds for plant embryo.

Enamel. See "Teeth" (p. 214).

Endothelium. See "Epithelia" (p. 151).

Entire Animal Objects. See "Animal Tissue" (p. 116) and "Aquatic and Marine Organisms" (p. 128).

Epichloë.

Treat as under "Peronosporaceæ" (p. 191).

Epidermis of Frog. See "Epithelia" (p. 151).

Epidermis of Leaf (Fresh).

- (i) 1° Macerate thin leaves in sodium hydroxide (5% or weaker), or boiling water. (1 min.).
- 2° Strip epidermis from under-side of leaf, and, with a camel-hair brush, remove debris.
- 3° Wash twice in distilled water.

Either (a),

- 4° *St.* eosin Y aq.
- 5° *Mt.* acetic acid (2%), or glycerine, or glycerine jelly.
- 6° Ring.

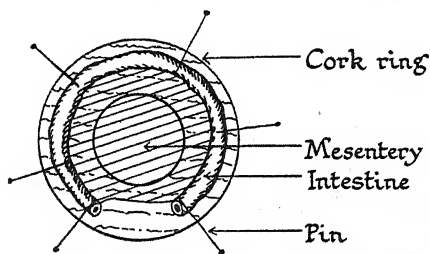
Or (b),

- 4° *St.* Safranin O or Delafield's hæmatoxylin.
- 5° Dehydrate.
- 6° Clear.
- 7° *Mt.* Canada balsam.

- (ii) See "Plant Tissue," (i), (v) (b), and (viii) (pp. 193-4), and Methods T-1 (p. 74), and T-2 (p. 75).

Epithelia.

- (i) *To prevent curling.* Stretch thin epithelia over a glass tube (and tie on), or stretch over a cover-slip.
- (ii) *Fix.* Weak formol-saline.
- (iii) *St. Perm.* Acetic acid (1%) (nuc.); Delafield's hæmatoxylin; crystal violet; methylene blue; van Gieson's (\rightarrow yellow).
- (iv) *To show stomata in fresh pavement epithelium.*
 - 1° Place in silver nitrate (0.5%). (5 mins.).
 - 2° Rinse in distilled water.
 - 3° Expose to sunlight.
 - 4° *Mt.* Glycerine.
- (v) *To show nuclei in squamous epithelium (e.g. epithelium inside cheek).*
St. Acetic acid (1%) or iodine.
- (vi) *To show cement substance (cell outlines) in frog mesentery.**
 - 1° Open up a fresh frog and remove a large coil of the intestine.
 - 2° Pin the coil to a cork ring so that the mesentery is stretched out taut across the ring (Fig. 19).



[J. Carpenter.]

FIG. 19.—Method of stretching frog mesentery on cork ring.

- 3° Rinse with sodium nitrate (1.5% aq.) (to remove surface chlorides).
- 4° In the dark, immerse in silver nitrate (0.5% aq.). (5–20 mins.).

* From J. G. Hawkes.

5° In the dark, rinse in distilled water.

6° Either, (a) expose to sunlight (to reduce silver nitrate).

or, (b) immerse in amidol (1%), and wash in distilled water.

(5 mins. : not too long, or tissue blackens).

7° Cut out a section of the now stiff mesentery.

8° Dehydrate.

9° Clear in xylene.

10° *Mt.* Canada balsam.

N.B. Carry out processes in watch-glasses and agitate frequently.

(vii) See also Methods T-4 (p. 77) ; T-5 (p. 78) ; P-2 (p. 82) ; and P-4 (p. 85).

Erythrocytes. See "Blood," pp. 133 ff.

Euglena.

(i) See "Protozoa" (p. 202), but difficult by ordinary methods ; best treated as for delicate algæ. See "Algæ" (iv) (p. 113).

(ii) *Mt.* Direct in Berlese's fluid or glycerine jelly, unless a method for algæ is being used.

(iii) *To see flagellum.*

Irrigate under cover-slip with very dilute iodine.

Eye.

(i) *Crayfish eye.* [See "Astacus" (iv), p. 129.]

(ii) *Vertebrate eye.* Owing to the complicated structure of this organ it is not a useful subject for beginners. More advanced workers should refer to Carleton, *Histological Technique*. Before preservation, fix in Müller's fluid.

Fasciola.

(i) See "Planaria" (p. 193) and "Tænia" (p. 213).

(ii) *Fix.* Alcohol (70%). Press between two slides tied together with cotton.

- (iii) *To show excretory and alimentary system.*
- 1° Inject a dilute medium (see "Injection Fluid," p. 254) through body wall at posterior end with a fine hard-glass pipette.
 - 2° Harden in alcohol (90%). Keep pressed between slides.
 - 3° Dehydrate.
 - 4° Clear clove oil. (*Several weeks*).
 - 5° *Mt.* Canada balsam.
- (iv) *Whole Mount.*
- (a) *St.* Borax carmine. Prolonged overstain and differentiate.
 - (b) *Clear and Mount.* As (iii) 4° and 5° (above).
- (v) *Sections.* Quite thin sections of hardened specimens can be cut with a razor, free-hand, if the specimen is held between two pieces of carrot.
- St. Mallory's; Delafield's hæmatoxylin.*

Fat.

- (i) *In animal tissue.*
- (a) *St.* Sudan III (see below); osmium tetroxide (2%) (→ grey or black).
 - (b) N.B. The method * given will show unmasked fats but not masked fats. If sections are required they must be made by the freezing method. (See advanced texts in Bibliography, p. 323).
- 1° Tease tissue in a small quantity of saline on the slide.
 - 2° Fix in vapour of formaldehyde (40%). ($\frac{1}{2}$ –1 min.).
 - 3° Flood slide with alcohol (50%). (3 mins.).
 - 4° *St.* Sudan III in a covered Petri dish. ($\frac{1}{2}$ –1 hour).
 - 5° Wash alcohol (50%).
 - 6° *Mt.* glycerine (50%); or glycerine jelly. (Fat globules → red.)

* After Carleton, *Histological Technique*.

(ii) *In plant tissue.*

St. Alkannin (\rightarrow red); osmium tetroxide (2%)
(\rightarrow brown-grey-black).

Feather.

- 1° Place in alcohol (100%). (Few mins.).
- 2° Dealcoholize in benzene-phenol.
- 3° *Mt. Canada* balsam.

Fern Frond.

Do not fix before cutting sections.

Fibres (Textile and Vegetable).

- 1° Dehydrate to alcohol (90%).
- 2° Clear in terpeneol.
- 3° Mount in *Canada* balsam (or in "Euparal" which, being of lower refractive index, enhances visibility).

Fish.

- (i) *Kill and fix.* Formaldehyde (10%).

Fish Scales.

- (i) 1° Wash and dehydrate to alcohol (100%).
 - 2° Clear in cedar-wood oil.
 - 3° Pass quickly through xylene.
 - 4° *Mt. Canada* balsam.
- (ii) *Mt.* Direct in glycerine jelly.

Flagella.

St. Carbol fuchsin; iodine.

Flustra. See "Polyzoa" (p. 201).

Fresh Tissue. See "Animal Tissue" (p. 116), and "Plant Tissue" (p. 193).

Fresh-Water Mussel. See "Anodon" (p. 127).

Frog. See "Rana" (p. 204).

Frozen Sections. Refer to more advanced books suggested in Bibliography, p. 323.

Fucus.

- (i) See "Plant tissue," viii, I (b) (p. 195).
- (ii) *Sexual organs.*
 - (a) *To castrate to empty.*
 - 1° Hang the plant in air. (6 hours).
 - 2° Replace in sea water. (6 hours).
 - 3° And so on.
 - (b) *Fix.* Osmium tetroxide (1%) ; bromine vapour ; boiling water.
 - (c) *Harden.* Alcohol (70%).
 - (d) *St. Sections.* Crystal violet ; Delafield's hæmatoxylin ; borax carmine.
 - (e) *Clear.* Clove oil.
 - (f) *Mt.* Canada balsam.
 - (g) *Wax-embedded Sections.* See Method PW-3 (p. 109).
- (iii) *Spermatozooids.*
 - (a) Tease out in clove oil with two needles, and clear.
 - (b) *St.* Iodine and picric acid.

Fungi.

- (i) *Fresh specimens.**
Examine by reflected light (p. 58) with a 1" or $\frac{2}{3}$ " objective.
- (ii) *Fresh specimens on agar.**
Place a block of agar on the slide. Examine as (i).
To obtain a lateral view, cut a thick slice of agar and lay it on its side on the slide.
- (iii) *Temporary preparations.**
 - (a) *Specimens containing air.*
Either (1) 1° Dip in alcohol (70%) or (90%) to remove air.
2° Wash in water.
3° Mount in water, or in dilute glycerine.
or (2) Mount direct in glacial acetic acid.

* Adapted from *The Structure and Development of the Fungi*, Gwynne-Vaughan and Barnes ; Cambridge University Press.

(b) *Moist specimens: asci.*

Mount direct in water, or in dilute glycerine.

(iv) *Permanent preparations.*

N.B. It is essential to fix and (unless the fixative contains alcohol) to remove air from the tissue as soon as the specimen is collected. Best results are generally obtained by collecting in the afternoon. Material should be cut in pieces not bigger than 0.5 cm. in any direction.*

(a) *Hand-sections of fleshy fungi for general characters and habit: loose hyphae: hyphae and fructifications.*

- (1)* 1° Either, fix in acetic-alcohol (15-30 mins.) and wash in alcohol (90%), or (95%) (2-3 hours) and omit 3° (below);

or, fix in alcohol (70%), and wash thoroughly in water;

or, fix in chrom-acetic, and wash in running water (6-12 hours).

2° Section if necessary.

3° Dehydrate in alcohol (30%) and (50%).

4° Transfer to a mixture of

Glycerine (pure)	. 1 part.
Either, erythrosin bluish (aq.)	. 1 part.
or, aniline blue W.S. (aq.)	. 1 part.
(Few minutes).	

5° Wash in glycerine (50% aq.) to remove excess stain.

6° Transfer to fresh glycerine (50% aq.) and allow to concentrate in the air.

7° Mount in glycerine-jelly (p. 45).

[If the specimen is on agar, cut away all but a thin slice of agar. On transference to warm glycerine jelly the agar will melt, and the specimen will clear.]

* *loc. cit.*

- (2) 1° Fix in alcohol (70%).
- 2° Wash in water.
- 3° Section if necessary.
- 4° Stain either, in crystal violet, or in Ziehl's carbolic-fuchsin.
- 5° Wash in distilled water.
- 6° Transfer either, to glycerine (10%),
or, to a solution consisting of

Distilled water	5 gms.
Industrial spirits [= alcohol (95%)]	4 gms.
Glycerine	1 gm.
- 7° Allow the glycerine, or the solution, to concentrate by evaporation at room temperature. (*Several days*).
- 8° Mount in pure glycerine (p. 44), or in glycerine-jelly (p. 45).

- (3) 1°-3°, as (iv) (a) (2) 1°-3°, above.
- 4°-5°, as (iv) (a) (2) 6°-7°, above.
- 6° Stain and mount by placing in glycerine jelly containing safranin O.
(*The stain passes into the fungus in the course of a week*).

(b) *Wax-embedded sections for detail work, e.g. cytology, spores, spore-mother cells.**

N.B. Material may be stored in Calberla's fluid after process 9°.

Unless nuclear detail is required, processes 4°, 6°, and 8° are to be omitted.

Unless the material is delicate, processes 12° and 14° are to be omitted.

- 1° Fix in Flemming's fluid (stock solution diluted to 50% with distilled water).
- 2° Wash in running water. (6-12 hours).
- 3° Dehydrate in alcohol (20%). (1 hour).

* *loc. cit.*

- [4° Dehydrate in alcohol (30%). (3 hours).]
- 5° Dehydrate in alcohol (40%). (3 hours).
- [6° Dehydrate in alcohol (50%). (3 hours).]
- 7° Dehydrate in alcohol (60%). (3 hours).
- [8° Dehydrate in alcohol (70%). (3 hours).]
- 9° Dehydrate in alcohol (80%). (3 hours).
- 10° Dehydrate in alcohol (90%). (3 hours).
- 11° Dehydrate in alcohol (100%). (3 hours).
- [12° Transfer to mixture of
 - Alcohol (100%) . . 75%
 - Chloroform . . . 25% (3 hours)].
- 13° Transfer to mixture of
 - Alcohol (100%) . . 50%
 - Chloroform . . . 50% (3 hours).
- [14° Transfer to mixture of
 - Alcohol (100%) . . 25%
 - Chloroform . . . 75% (3 hours).]
- 15° Transfer to chloroform in an uncorked capsule (2 cms. diam. \times 3 cms. long). Add a few shavings of paraffin wax (m.pt. 52° C.) (to press the material into the chloroform) and keep at room temperature. (3 hours).
- 16° Place the capsule *on* the oven and, as the chloroform evaporates, add further paraffin wax from time to time. (3 hours).
- 17° Place the capsule *in* the oven (53° C.). (20 mins.).
- 18° Transfer contents of capsule to warm watch-glass in the oven, to allow all the chloroform to evaporate. (*Approx.* 10 mins.).
- 19° Warm a stoneware saucer, 2" diam., in the oven; smear the inside thinly and evenly with glycerine; fill it with fresh molten paraffin wax; using warmed forceps, transfer the impregnated material to the wax, for embedding.

While the wax is still molten, insert a slip of paper with the name and treatment of the tissue written on the protruding part.

20° Solidify the paraffin wax quickly. Hold the vessel on a dish of water; blow on the surface to form a pellicle; finally immerse the vessel in cold water. If the inside of the vessel has been carefully coated with glycerine the block will float out of it. If it does not, it must be cut out round the edges.

21° Cut sections at 10μ to 15μ (p. 92).

22° Attach sections to slide (p. 95).

(For detail work be careful to keep the sections in sequence.)

23° Remove wax from sections (p. 96).

24° Wash off xylene with alcohol (100%).

25° Wash in alcohol (90%).

26° Transfer to distilled water.

[Sections *attached to the slide* will not be harmed by transference from alcohol (90%) to water.]

27° Stain according to one of the following methods:

Either,

(1) *For general work: and for parasitic fungi in host tissue: **

Note: The methylene blue and erythrosin bluish combination is useful if the host's tissues are very resinous.

28° Stain either in safranin O. or in methylene blue aq. (5 mins.).

29° Wash in distilled water. (2 mins.).

30° Dehydrate in industrial spirit [= alcohol (95% approx.)]. (2 mins.).

* *loc. cit.*

METHODS FOR FUNGI

- 31° Dehydrate in alcohol (100%). (2 mins.)
 32° Counterstain either, in light green S.F.-yellowish (in clove oil) (watch under microscope until safranin O remains only in nuclei and in lignified walls of host); or, in erythrosin bluish (satd. soln. in clove oil).

33° Wash in xylene.

34° Mount in Canada balsam.

(Results with safranin O and light green S.F.-yellowish: hyphae → green; cellulose walls of host → green; nuclei → red; lignified walls of host → red. Results with methylene blue and erythrosin bluish: hyphae → red; cellulose walls of host → red; nuclei → blue; lignified walls of host → blue.)

Or,

(2) *For general work, especially with dense storage substances: **

28° Stain in crystal violet (aq.). (10 mins.).

29° Wash in water.

30° Dehydrate in industrial spirit [= alcohol (95% approx.)]. (Rapidly).

31° Dehydrate in alcohol (100%). (Rapidly).

32° Counterstain in light green S.F.-yellowish (in clove oil).

33° Wash in xylene.

34° Mount in Canada balsam.

(Chromatin → purple; cellulose walls → green; cytoplasm → almost colourless.)

Or,

(3) *For general cytological characters:*

(A) Heidenhain's hæmatoxylin counterstained with light green S.F.-yellowish or erythrosin bluish, or orange G.*

* *loc. cit.*

- 28° Mordant in iron alum.
(20 mins.-2 hours).
- 29° Wash in distilled water.
- 30° Stain in Heidenhain's hæmatoxylin.
(1-24 hours).
- 31° Wash thoroughly in distilled water.
- 32° Differentiate rapidly, under the microscope, in a Petri dish containing iron alum (2%-8% in distilled water) to which 3-4 drops of glacial acetic acid have been added.
- 33° Wash in distilled water.
- 34° Wash in running tap-water to remove acidity. (5 mins.).
- 35° Dehydrate in industrial spirit [= alcohol (95% approx.)].
- 36° Dehydrate in alcohol (100%).
- 37° Counterstain either, in light green
S.F. yellowish, in
clove oil;
or, in erythrosin bluish, in
clove oil;
or, in orange G, in clove
oil.
- 38° Wash in xylene.
- 39° Mount in Canada balsam.

(B) Newton's crystal violet method.

See "Mitotic Figures" (iii) (b) (p. 179).

(C) Flemming's triple stain.

(Crystal violet, safranin O, and orange G).

See "Mitotic Figures" (iii) (e) (p. 180).

Or,

(4) *For cytological work with large nuclei* : *

Breinl's stain.

(Safranin O, polychrome methylene blue, and orange tannin.)

* *loc. cit.*

- 28° Place in iodine (special formula, p. 256).
(15 mins.).
- 29° Wash in water.
- 30° Stain in safranin O (special formula,
p. 281). (30 mins. or longer).
- 31° Wash in water.
- 32° Stain in polychrome methylene blue
(special formula, p. 273). (10 mins.).
- 33° Wash in water.
- 34° Place in orange tannin.
(*Watch under microscope: until orange
displaces blue in cytoplasm.*)
- 35° Wash in water.
- 36° Wash in alcohol (95%). (*Quickly*).
- 37° Wash in alcohol (100%). (*Quickly*).
- 38° Differentiate in aniline oil.
(*Use commercial aniline oil—pure oil
not effective.*)
- 39° Transfer to cedar-wood oil.
- 40° Examine sections under H.P. and select
the best for balsam mounting.
- 41° Pass quickly through xylene.
- 42° Mount in Canada balsam.
(*Spireme → brilliant blue-black; cyto-
plasm → faint yellow; chromosomes of
meiotic metaphase → bright red.*)

(c) *Parasitic hyphae in host plant.*

(1) *Durand's Method.**

- 1° *St.* sections deeply in Delafield's hæmatoxylin.
- 2° "Blue" in alkaline water.
- 3° Wash alcohol (95%).
- 4° Dehydrate alcohol (100%).
- 5° Clear carbol-turpentine.

* After E. J. Durand, *Phytopathologist*, I, p. 129, quoted by Strasburger, *Handbook of Practical Botany* (English Edn.), Allen and Unwin.

- 6° *St.* sections eosin Y [1.5% in alcohol (95%)].
(5-10 mins.).
- 7° Place sections on slide.
- 8° Remove excess stain with filter-paper.
- 9° Wash thoroughly carbol-turpentine (*not* alcohol). (Use a pipette for this process.)
- 10° Wash xylene.
- 11° *Mt.* Canada balsam.
(Host's tissues → blue ; parasitic hyphae → red.)
- (2) *Godwin's Method.**
- 1° Place section on slide in a drop of lacto-phenol (special formula, p. 259).
- 2° Heat over a bunsen flame.
(*Few seconds, till lacto-phenol fumes.*)
- 3° Transfer section to aniline blue W.S. in lacto-phenol (special formula, p. 227).
- 4° Warm again on the slide.
- 5° Transfer to lacto-phenol, and heat and examine alternately.
(*To remove excess stain.*)
- 6° Mount in fresh lacto-phenol.
- 7° Cover.
- 8° Ring.
- (3) 1° Wash sections in alcohol (30%).
- 2° Stain in Pianese IIIb. (15-45 mins.).
- 3° Wash in water. (*To remove excess stain.*)
- 4° Differentiate in
Alcohol (95%) . . . 100 c.c.
Hydrochloric acid (conc.) . 1.0 c.c.
- 5° Dehydrate alcohol (70%), (90%), (100%).
- 6° Clear benzene-phenol.
- 7° *Mt.* Canada balsam.
(Parasitic hyphae → deep pink ; host tissue → green.)

* With acknowledgments to Dr. H. Godwin.

- (4) As (iv) (b), staining by method (iv) (b) (1) (p. 159).
- (5) See also "Hymenomycetes" (p. 169), "Peronosporaceæ" (p. 191), "Rusts" (p. 207), and "Stereum purpureum" (p. 212).

(d) *Structures with food supplies* * (e.g. *Pyronema oogonia*):

- 1° Fix in Merkel's fluid.
- 2° Wash in running water. (6-12 hours).
- 3° Proceed as from process 3° of (iv) (b) (p. 157).

(e) *Very minute objects* : *

(Handling in dehydration and impregnation undesirable).

- 1° Fix and wash as (iv) (b), processes 1° and 2° (p. 157).
- 2° Transfer to glycerine (10% aq.) and allow to concentrate to pure glycerine in a desiccator. Test purity by adding a drop of pure glycerine.
- 3° Wash thoroughly in alcohol (100%).
- 4° Transfer to a capsule containing a mixture of
 Alcohol (100%) 90 parts.
 Cedar-wood oil 10 parts.
- 5° Allow the alcohol to evaporate at room temperature. (*Till only the oil remains*).
- 6° Add shavings of paraffin wax (m.pt. 52° C.) at room temperature. (3 hours).
- 7° Place the capsule *on* the oven and add more paraffin wax from time to time. (3 hours).
- 8° Place the capsule *in* the oven (53° C.). (20 mins.).
- 9° Transfer to pure paraffin wax in oven. (5 mins.).

* Adapted from *The Structure and Development of the Fungi*, Gwynne-Vaughan and Barnes; Cambridge University Press.

10° Transfer to fresh pure paraffin wax in oven.
(5 mins.).

(Changes are to ensure that no cedar-wood oil is carried into the embedding wax.)

11° Proceed as from process 19° of (iv) (b) (p. 158).

(f) *Delicate specimens* :

See "Algæ" (iv) (p. 113).

(v) See also "Agaricus" (p. 112), "Mucor" (p. 183),
"Penicillium" (p. 190).

(vi) *Spores*. Examine in water.

(vii) *To identify chitin*. See "Chitin" (p. 142).

(viii) *Cultures*. See "Fungi" (p. 302).

Glochidium. See "Anodon" (iii) (p. 127).

Glycogen.

(i) *To precipitate (in water sol. form)*. Alcohol (not weaker than 50%).

(ii) *St.*

(a) *Temp.* Iodine.

(b) *Perm.* To preserve the stain in permanent preparations, after staining with iodine, treat with potassium acetate (aq. satd.). Ring cover-slip with gold size.

(c) *Method for wax-embedded sections.*

1° Float sections on to slide with alcohol.

2° Dry in oven (37° C.). (Overnight).

3° Place in celloidin (1%). (Overnight).

4° Drain and allow to dry.

5° Remove wax with xylene.

6° Wash off xylene with alcohol (100%).

7° Transfer to alcohol (80%).

8° Wash in distilled water.

9° *St.* Carazzi's hæmatoxylin.

10° Wash in tap-water.

11° Stain in Best's carmine.

12° Differentiate in Best's differentiator.

(*Till no more stain removed*).

13° Dehydrate in alcohol (90%).

14° Dehydrate in xylene-acetone.

15° Clear in xylene.

16° *Mt.* Canada balsam.

Golgi Bodies.

Modified Cajal's method.

1° Cut tissue in pieces not more than 5 m.m. thick (preferably less).

2° Fix (within 3 hours of death) in Cajal's fixative (de Fano's modification). (3-8 hours).

3° Rinse in distilled water.

4° Transfer to silver nitrate at room temperature. [Solution (ii) (p. 283).] (36 hours).

5° Wash twice in distilled water. (*Rapidly*).

6° Reduce in hydroquinone. [Mixture (i) (p. 254).] (9-12 hours).

7° Wash in running water. (5 mins.).

8° Dehydrate in alcohol (30%), (50%), (70%), (90%), (100%). (*Not more than 2 hours each*).

9° Clear.

10° Impregnate and embed (pp. 89 ff.).

11° Cut sections at 4μ (p. 93).

12° Fix sections to slide (p. 95).

13° Remove wax with xylene (p. 96).

14° Wash in alcohol (100%). (*Rapidly*).

15° Either,

a° Clear in xylene.

b° *Mt.* in Canada balsam.

Or,

A° Hydrate in alcohol (95%).

B° Hydrate in distilled water.

C° Tone sections in toning solution (p. 286).
(*Watch under microscope, 5-10 mins.: cytoplasm from yellowish \rightarrow grey.*)

D° Wash in running water.

[E° If desired, counterstain in Delafield's hæmatoxylin.]

[F° "Blue" in tap-water.]

G° Dehydrate in alcohol (95%). (*Rapidly*).

H° Dehydrate in alcohol (100%). (*Rapidly*).

I° Clear in xylene. (*Rapidly*).

J° *Mt. Canada* balsam.

Cytoplasm → yellow - brown (if untoned), or → grey (if toned); Golgi bodies → black (network or rodlets); Mitochondria → impregnated golden-brown.

Gums.

St. Hanstein's rosaniline violet (→ red).

Hair.

(i) *St.* Picric acid aq. or alc.

(ii) See "Skin" (p. 209).

Heart. See "Blood Vessels" (p. 137).

Helianthus.

See Methods T-6 (p. 79); T-7 (p. 80); P-1 (p. 81); and P-5 (p. 86).

Helix.

(i) *To kill.*

(a) *To obtain in an expanded condition.*

Either, place in a screw-top bottle brimful of tepid, boiled water and screw down the top.

Or, *place in a beaker of cold water, heat gently till expanded, then heat quickly (without cooling) for 1 min.

(b) Place in mercuric chloride (sat. aq.). ($\frac{1}{2}$ hour).

When dead wash under running water very thoroughly.

(ii) *To remove mucus.*

When dead, wash in alcohol (50%).

* *Gerrard's Bulletin*, May 1939. T. Gerrard & Co.

- (iii) *Radula and jaw plate.*
St. Delafield's hæmatoxylin.
- (iv) *Salivary gland.*
St. Borax carmine.
- (v) *Nerve collar.*
 - (a) *St.* Borax carmine.
 - (b) *Mt.* Under cover-slip raised on glass ledges.

Hirudo.

- (i) *Kill for dissection.* Chloroform vapour, or alcohol (30%).
- (ii) *Fix.* Alcohol (60%).
- (iii) *For sectioning.*
 - (a) *Narcotize.* Two menthol crystals placed on small water surface. (*Till immobile*).
 - (b) *Fix.* Bouin's fluid. (18 hours).
 - (c) *St.* Mallory's; Delafield's hæmatoxylin.

Hormidium.

- (i) See "Algæ" (p. 112).
- (ii) *St.* Delafield's hæmatoxylin.

Horn.

- (i) *St.* Picric acid alc. or aq.
- (ii) See also "Skin" (p. 209).

Hydra.

- (i) *St. living.* Nile blue sulphate.
- (ii) *To protrude nematocysts.*
 - (a) Irrigate with sodium chloride (5% aq.); dilute iodine; dilute acetic acid.
 - (b) *St.* Acetic-methylene blue.
- (iii) *To kill.*
 - (a) Hot Bouin's fluid.
 - (b) See "Coelenterates" (p. 144).
- (iv) *To see cell structure.*
Kill and macerate in acetic acid (1%) with a trace of osmium tetroxide. (5 mins.).
Disintegrate with small camel-hair brush.

- (v) *To obtain in an expanded condition.*
- (a) Place first in a little cold water : then add boiling water.
 - (b) Narcotize slowly with menthol.
 - (c) Suddenly apply Bouin's fluid, or formaldehyde (10%), or mercuric chloride (satd. aq.), all at 60° C. ; Perenyi's fluid (see p. 270).
- (vi) *Fix.* Bouin's fluid.
- (vii) *Permanent preparations.*
- (a) *Whole mount.*
See " Animal Tissue " (v) General histology.
Method I (a), p. 117.
 - (b) *To stain nematocysts in a whole mount.*
 - 1° Kill with mercuric chloride (satd. aq.) at 60° C.
 - 2° Wash thoroughly in alcohol.
 - 3° *St.* very strongly with methylene blue.
 - 4° Dehydrate rapidly.
 - 5° Clear cedar-wood oil.(Nematocysts → blue ; rest of body → unstained).
 - (c) *St. sections.*
Delafield's hæmatoxylin ; acetic-methylene blue.
 - (d) *Mt.* (α) Glycerine ; balsam ; or formaldehyde (5%). (Ring if necessary.)
(β) See also " Cœlenterates " (iii) (a), p. 144.

Hydractinia. See " Cœlenterates " (p. 144).

Hydroids.

- (i) See " Cœlenterates " (p. 144).
- (ii) Proceed as for " Echinus " (ii) (p. 149).

Hymenomycetes.*

Material for nuclear division is best secured at midnight.
Treat as for " Fungi " (iv) (b) (p. 157).

* After Gwynne-Vaughan and Barnes, *Structure and Development of the Fungi*, Cambridge University Press.

Insects.

- (i) *Small.*
 - (a) *Kill*: Ethyl acetate, or acetic-alcohol.
 - (b) *Preserve*: Ethyl acetate (keeps muscles relaxed), or alcohol (70%).
 - (c) *Permanent Whole Mount.*
 - 1° Kill and clear in Berlese's fluid (p. 229).
 - 2° Mount in Berlese's fluid.
 - 3° Dry off in oven.
 - 4° Ring.
 - (d) *Mouth Parts*:
 - 1° Boil the whole head in potassium or sodium hydroxide (10%). (5 mins.).
 - 2° Wash in water. (2 mins.).
 - 3° Dehydrate in alcohol (30%), (50%), (70%), (90%), (100%). (2 mins. each).
 - 4° Clear in xylene.
 - 5° Either (α) Mount whole head in Canada balsam.
 - or (β) Remove mouth parts and mount separately.
 - (e) *Parts of small insects.*
 - 1° Dehydrate up to alcohol (90%).
 - 2° Proceed as for "Echinus" (ii) (p. 149) from process 7° onwards.
 - (f) See also under "Acarine" (p. 111).
- (ii) *Medium or Large.*
 - (a) *Kill*: Ethyl acetate; laurel or cyanide (see "Killing Bottle," p. 258); chloroform.
 - (b) *Preserve*: Ethyl acetate (keeps muscles relaxed), or by drying.
 - (c) *Mouth parts and other organs*:
See "Periplaneta" (p. 191).
- (iii) *Larvæ.*
 - (a) *Small*: Treat as whole insect mounts (i) (c) above.
 - (b) *Large, e.g. Caterpillar*:
 - 1° Boil in dilute potassium or sodium hydroxide (2%). (3-4 mins.).

- 2° Wash in water. (10 mins.).
- 3° Cut in half lengthwise.
- 4° Dehydrate in alcohol (30%), (50%), (70%), (90%), (100%). (10 mins. each).
- 5° Clear in cedar-wood oil. (Till transparent).
- 6° Wash in xylene.
- 7° Mount in Canada balsam.

Intestine. See "Alimentary Canal" (p. 115).

Inulin.

(i) *To find:*

Soak dandelion (*Taraxacum officinale*) roots in alcohol (70%) the night before examination.
Crystals small and spherical.

(ii) 1° *St.* Orcein (alc.).

2° Boil in dil. hydrochloric acid. (Inulin → orange red.)

Iron.*

Iron compounds in sections.

- 1° Wash in acid alcohol.
- 2° Wash in alcohol (90%).
- 3° Wash in distilled water.
- 4° Place in potassium ferrocyanide (p. 273).
- 5° Wash in distilled water.
- 6° Stain in eosin Y [see (e), p. 245]. (3 mins.).

Jellyfish.

(i) *Kill.* Alcohol (70%).

(ii) *Fix.* Formaldehyde (10%).

Keratin.

(i) *Fix.* Formol-saline.

(ii) *St.*† Hæmatoxylin and eosin Y (→ bright pink); iron hæmatoxylin and van Gieson's (→ black); Mallory's (→ bright red).

* After Bolles-Lee, *Microtometist's Vade-Mecum*.

† After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

Kidney.

- (i) *Fix.** Do not fix in Bouin's fluid. Use formol-saline or "corrosive-formaldehyde."
- (ii) *St.†* (a) Ehrlich's hæmatoxylin (counterstained with eosin Y alc.).
- (b) Delafield's hæmatoxylin [counterstained with eosin Y aq. (see p. 119)].

Latex.

- (i) *Protein granules.* *St.* Iodine.
- (ii) *Starch* *"* *St.* Iodine.
- (iii) *Rubber* *"* *St.* Alkannin (→ red).

Leaf. See "Plant Tissue" (p. 193), and "Mesophyll" (p. 177).

Leech. See "Hirudo" (p. 168).

Lens Cells. See "Astacus" (iv) (p. 129).

Leucocytes (invertebrate). See "Blood" (iii) (1) (b) (p. 133).

Lichens.

St. Cyanin and erythrosin bluish. (Algæ → blue; fungus filaments → red.)

Ligament.

- (i) *Fix.* Formol-saline (4%).
- (ii) *Embed.* Hardly sufficient rigidity with paraffin. Celloidin method required. See Bibliography (p. 323).
- (iii) *St.* van Gieson's; Weigert's stain; Mallory's.

Lignified Tissue. To Distinguish Venation in. See "Bundle Ends" (p. 138).

* After Carleton, *Histological Technique*, Oxford Medical Publications.

† After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

Lignin.(i) *St.* (a) *Temp.*

Aniline sulphate or chloride (specific stains) (\rightarrow yellow); corallin (\rightarrow deep red); phloroglucin (soak tissue and add HCl conc.) (specific stain) (\rightarrow violet-red).

(b) *Perm.*

Loeffler's methylene blue (counterstained with eosin Y); safranin O (counterstained with Delafield's hæmatoxylin, or aniline blue W.S., or light green S.F. yellowish in clove oil); cyanin (\rightarrow blue) (counterstained with Bismarck brown Y); iodine green (counterstained with Mayer's carmalum, or acid fuchsin, or eosin Y, or erythrosin bluish); Hanstein's rosaniline violet (\rightarrow reddish); basic fuchsin (\rightarrow red); methyl green (\rightarrow green); xylene-methylene blue (\rightarrow blue); xylene-nile blue sulphate (\rightarrow blue).

(ii) See "Xylem" (p. 217).

Limpet. See "Patella" (p. 190).

Lipoids.

St. Osmium tetroxide (\rightarrow brown).

Liver.*

(i) *Fix.* Formol-saline (4%); "corrosive-formaldehyde."

(ii) *Section* at 8μ – 10μ .

(iii) *St.* (a) *Nuclei in fresh teased liver.* Acetic acid (1%).

(b) *General histology.* See p. 117.

(c) Ehrlich's hæmatoxylin and eosin Y alc.; Heidenhain's iron hæmatoxylin (liver cells \rightarrow dark; canaliculi \rightarrow unstained tubules).

* Except (iii) (a) and (b), after Carleton, *Histological Technique*, Oxford Medical Publications.

Liver Fluke. See "Fasciola" (p. 152).

Living Organisms.

- (i) *St.* Methylene blue aq.; Janus green B; methyl green; neutral red; Bismarck brown Y.
- (ii) *Fixing, etc., small.* See "Protozoa" (p. 202).

Lobworm. See "Arenicola" (p. 128).

Lugworm. See "Arenicola" (p. 128).

Lumbricus.

- (i) *Kill.* Chloroform vapour; alcohol (30%) (inclined to harden unduly); drop into hot (not boiling) water, or hot sodium chloride (20% aq.); chrom-acetic (12 hours for fixation).
- (ii) *Fix.* Alcohol (95%).
- (iii) *Whole specimens.* (Or typical parts of whole specimens.) Use small worms.
 - 1° *Kill.* Alcohol (30%).
 - 2° *St.* Borax carmine; or Delafield's hæmatoxylin followed by tap-water. (Also try unstained.)
 - 3° *Clear.* Cedar-wood oil.
 - 4° *Preserve.* Cleared specimen in a tube of cedar-wood oil.
- (iv) *Ovary.** (From fresh specimen.)
 - 1° Dissect out and examine in water. It is easier for the beginner to detach the ovary with a piece of septum, and to use the septum for handling with the forceps.
 - 2° Remove all traces of extraneous tissue.
 - 3° By means of a pipette, transfer to two drops of glycerine (50% aq.). (5 mins.).
 - 4° Add glycerine (pure) drop by drop. (15 mins.).
 - 5° Prepare a paper frame (see p. 41) and soak it first in water and then in glycerine.

* After Green, *School Science Review*, XV, 408, March 1934.

6° Transfer to pure glycerine enclosed by paper frame on slide.

7° Cover.

8° Ring with gold size.

Note : Preparations may be unstained, or stained in borax carmine or hæmatoxylin after 2° (above).

(v) *Seminal vesicles.*

(a) *Spermatozoa.* (See p. 209.) *St. Hofmann's* W.S. violet (0.25%).

(b) See "Monocystis" (p. 181).

(vi) *Nephridium.*

(a) *To demonstrate in a dissection.*

Flood the dissection either with mercuric chloride (sat. aq.); or with methylene blue [0.125% in sodium chloride (0.6% aq.)].

(10 mins.).

(b) *Permanent preparation.*

It is difficult to remove an entire nephridium. The beginner should detach one, *with a septum*, and hold the septum (not the nephridium) in the forceps.

1° Fix in alcohol (70%) (2 mins.).

2° Stain in borax-carmine. (2 mins.).

3° Dehydrate,
either, in alcohol (70%), (90%), (100%).
(2 mins. each).

or, in "Cellosolve." (2 mins.).

4° Transfer to fresh alcohol (100%) or "Cellosolve" in a small specimen tube.

5° Clear in cedar-wood oil. Hold the tube of dehydrating agent (from 4°) at an angle to the vertical. Very gently, pour cedar-wood oil down the side of the tube. As the specimen clears it will sink in the cedar-wood oil.

6° When specimen is clear, pipette off the bulk of the dehydrating agent and allow the remainder to evaporate.

7° Pass the specimen quickly through xylene.

8° Mount in Canada balsam.

(c) *Parasitic nematode in nephridium.* See "Rhabditis" (pp. 205, 310).

(vii) *Sections.* St. Delafield's hæmatoxylin.

(viii) *Wax-embedded sections.* See Method PW-1 (p. 98).

Lung.*

(i) 1° If possible obtain the lung from an animal killed by a blow on the head.

2° Ligature trachea of dead animal and dissect out all contents of thoracic cavity in one piece.

3° Fix all contents in "corrosive-formaldehyde."
(12-48 hours).

4° Wash fixative out in usual way, cut lung into pieces ($\frac{1}{2} \times \frac{1}{2} \times 1$ cm.) and place in alcohol (70%) covered by pad of cotton wool. (*Many days.*) The object is to get rid of air.

(ii) St. Heidenhain's iron hæmatoxylin and van Gieson's.

Lymph Glands.*

(i) *Fix.* Zenker-formol.

(ii) St. Mallory's (retiform connective tissue → blue).

Lymphocytes. See "Blood" (p. 133).

Mammary Gland. See "Animal Tissue" (p. 116).

Medusæ. See "Coelenterates" (p. 144).

Mesentery, Cement Substance in Cell Outlines. See "Epithelia" (vi) (p. 151).

* After Carleton, *Histological Technique*, Oxford Medical Publications.

Mesophyll of Leaf.

- 1° Boil leaf in potassium hydroxide (10% aq.).
(5 mins.).
- 2° Wash in water.
- 3° Tease in drop of glycerine (50%) on slide.
- 4° Remove lumps and drain off glycerine.
- 5° *Mt.* Glycerine jelly.

Mite on Anodon Gill. See "*Atax ypsilophorus*" (p. 130).

Mitochondria.

- (i) *Fix.* Zenker-formol.
- (ii) *St.* Heidenhain's iron hæmatoxylin.
- (iii) Follow the method for Golgi bodies (p. 166) but, at process 6°, reduce in hydroquinone [mixture (ii) (p. 254)] for 6 hours.

Mitotic Figures.

- (i) *Fix.* Bouin's fluid; Zenker's solution; see also "Fungi" (iv) (b) (p. 157).
- (ii) *St.* Heidenhain's or Delafield's hæmatoxylin; crystal violet; Babe's safranin and light green S.F. yellowish.
- (iii) *In root tips of onion and bean.*
 - 1° Use young tips, not more than $\frac{1}{8}$ " long for good results. Carry out the operations in small glass tubes.
 - 2° Fix in Bouin's fluid (24 hours) under a filter pump to remove air from tips. (Removal of air takes about 2 hours.)
 - 3° Wash in running tap-water. (2 hours).
 - 4° Dehydrate in alcohol 10%, 20%, 50%, 75%, 95%, 100%, and fresh 100%. (2 hours each).
 - 5° Replace alcohol by xylene, using:
 - (a)° Alcohol (100%) 3 vols./xylene 1 vol. (2 hours).
 - (b)° Alcohol (100%) 2 vols./xylene 2 vols. (2 hours).

- (c)° Alcohol (100%) 1 vol./xylene 3 vols.
(2 hours).
- (d)° Xylene. (2 hours.)

6° Warm tubes to 40° C. in oven.

7° Impregnate

- (a)° Xylene 4 vols./wax, 1 vol. (6 hours).
 (b)° Xylene 2½ vols./wax, 2½ vols. (6-8 hours).
 (c)° Xylene 1 vol./wax, 4 vols. (6-8 hours).
 (d)° Pure wax. (6-8 hours).
 (e)° Fresh, pure wax—in an oven containing only tubes with pure wax. Remove corks from tubes to allow xylene to escape. (6-8 hours).

Note: A longer period in the wax tends to make the tips brittle and causes crumbling. Make all changes quickly, so that wax does not solidify.

8° Embed (p. 90).

9° Cut truly longitudinally at 10μ. Check orientation of block by examining the sections. Adjust block if necessary.

10° Attach sections to slide (p. 95).

11° Warm slide, dissolve off wax in xylene.

12° Wash in alcohol (100%), (90%), and distilled water (2 mins. each), *unless using method (d) (p. 180) when hydration is taken to alcohol (50%).* [Sections attached to slides are not harmed by being taken from alcohol (90%) to water.]

13° Stain.

Either (a)

14° Mordant in iron alum (2%). (30 mins.).

15° *St.* Heidenhain's hæmatoxylin. Be careful not to overstain. (20 mins.).

16° Wash in tap-water.

[If overstained:

a° Differentiate acid alcohol.

(*Watch under microscope.*)

b° Wash alcohol (70%). (1 min.).

c° "Blue" alkaline alcohol (90%).

d° Wash alcohol (90%). (1 min.).

17° Dehydrate in industrial spirit [= alcohol (95% approx.)], and in alcohol (100%).

(Quickly—by treatment on the slide).

18° Clear benzene-phenol.

19° *Mt.* Canada balsam.

Or (b) *Newton's Crystal Violet Method.*

14° Stain sections in crystal violet (1% aq.) which has previously been boiled and filtered.

(4-7 mins. according to nature of material.)

15° Wash in distilled water. (Several quick changes.)

16° Place in alcohol (80%) containing 1% iodine and 1% potassium iodide. (30 secs.).

17° Dehydrate alcohol (90%) and (100%), both containing iodine as in 16°. (Quickly).

18° Dehydrate alcohol (100%). (Quickly).

19° Clear and differentiate in clove oil.

20° Pass quickly through xylene to remove adherent clove oil.

21° *Mt.* Canada balsam.

Or (c) 14° Stain sections in crystal violet, (b) p. 238. (2 hours).

15° Transfer to Lugol's iodine. (2 mins.).

16° Dehydrate in alcohol (30%), (50%), (70%), (95%). (2 mins. each).

17° Transfer to acetone.

(Till colour ceases to come off.—Easy end point.) (2 mins.).

18° Transfer to alcohol (100%).

19° Clear in clove oil.

20° Pass quickly through xylene.

21° *Mt.* Canada balsam.

N.B. The above method (c) is not successful with animal tissues unless there is prolonged staining in crystal violet—the end point is difficult in differentiation.

- Or (d) 14° Stain in Babes's safranin. (24 hours).
 15° Hydrate in alcohol (50%). (2 mins.).
 16° Hydrate in alcohol (30%). (2 mins.).
 17° Transfer to Lugol's iodine. (2 mins.).
 18° Dehydrate in alcohol (30%), (50%), (70%), (95%). (2 mins. each.).
 19° Stain in light green S.F. yellowish [saturated solution in alcohol (95%)]. (*Till differentiated.*)
 20° Clear in cedar-wood oil.
 21° Pass quickly through xylene.
 22° *Mt.* Canada balsam.

- Or (e) *Flemming's Triple Stain.*
 14° Mordant in iodine. (5 mins.).
 15° *St.* crystal violet. (15 mins.).
 16° Wash alcohol (50%). (*Quickly.*)
 17° *St.* safranin O. (5 mins.).
 18° Dehydrate in industrial spirit [= alcohol (95% approx.)]. (*Rapidly.*)
 19° Dehydrate in alcohol (100%). (*Rapidly.*)
 20° Wash in a mixture of 50/50 :: xylene/alcohol (100%). (*Rapidly.*)
 21° Examine under the microscope.
 (Destaining is complete when chromosomes are red and spindle fibres are violet.)
 22° Transfer to clove oil.
 23° *St.* orange G in clove oil. (1 min.).
 24° Wash off excess stain in fresh clove oil.
 25° Wash in xylene.
 26° *Mt.* Canada balsam.

(Chromosomes → red; spindle fibres → violet; surrounding plasma → orange.)

(iv) See also "Rhabditis" (p. 205).

(v) See also "Fungi" (iv) (b) (p. 157), and stain by methods under (iv) (b) (3) (p. 160).

Molluscs. See "Aquatic and Marine Organisms" (p. 128), "Anodon" (p. 127); "Helix" (p. 167).

Monocot. Stem.

St. Hanstein's rosaniline violet.

Monocystis. (Adult and stages in life history.)

(i) See "Protozoa" (p. 202).

(ii) *Temp.*

Examine smears of seminal vesicles of earthworm in sodium chloride (0.75% aq.) on slide.

(iii) *Perm.*

Carry out all operations with cover-slip in covered watch-glasses.

(a) *1° Smear cut surface of seminal vesicle very thinly on cover-slip. Handle slip with forceps.

2° Allow almost to dry in the air.

3° Fix alcohol (70%-90%). (5 mins.).

4° *St.* Ehrlich's hæmatoxylin. (15 mins.).

5° Wash alcohol (70%).

6° Differentiate in acid alcohol.

(5-12 mins.—till pale reddish colour).

7° Wash alcohol (70%). (1 min.).

8° Wash alcohol (90%). (1 min.).

9° Blue in ammonia vapour, or alkaline alcohol.

10° Dehydrate alcohol (90%). (5 mins.).

11° Dehydrate alcohol (100%). (5 mins.).

12° Dehydrate alcohol (100%). (5 mins.).

13° Clear benzene-phenol. (2 mins.).

14° Mount. Invert slip in drop of Canada balsam on slide.

(b) See "Protozoa," (2) (iv) (p. 203).

(c) *Especially useful for Trophozoites.*

N.B. The cover-slip should be held in a slit in a match-stick, but *not* in the fingers or metal forceps.

1° Smear contents of seminal vesicle of freshly-killed earthworm, very thinly, on a clean cover-slip.

* After Green, *School Science Review*, XV, 408; March 1934.

METHODS FOR SPECIFIC MATERIAL

- 2° Fix in "corrosive-acetic" [solution (b) on p. 237] by *quickly* inverting the slip in the fixative. (2 mins.).
 - 3° Wash in distilled water. (2 mins.).
 - 4° Dehydrate in alcohol (30%), (50%). (2 mins. each).
 - 5° Remove fixative in alcohol (70%) to which a few drops of iodine solution have been added. If colour disappears add more iodine.
(*Till colour remains—about 2 mins.*).
 - 6° Dehydrate in alcohol (70%). (2 mins.).
 - 7° Stain in borax carmine. (10 mins.).
 - 8° Rinse in alcohol (70%). (*Quickly*).
 - 9° Differentiate in acid alcohol.
(*Care—till bright pink*).
 - 10° Wash in alcohol (70%).
(*Thoroughly—but quickly*).
 - 11° Dehydrate in alcohol (100%). (5 mins.).
 - 12° Clear in clove oil. (3 mins.).
 - 13° Pass through xylene. (*Quickly*).
 - 14° *Mt.* Canada balsam.
- [Pseudo-navicellae (→ unstained); larger trophozoites (→ pink with clearly defined nuclei); smaller trophozoites can be found in sperm morulae under high power.] *

Mosquito. See "Insects," (i) (p. 170).

Mosses.

Do not fix before sectioning. Stored material may be softened in glycerine.

St. Delafield's hæmatoxylin; Magdala red and aniline blue W.S.

Mucilage. See "Pectic compounds" (p. 190).

* Adapted from *School Science Review*, XVII, 66, 297; December 1935. From Department of Zoology, University of Glasgow.

Mucin.

*St.** Borrel's methylene blue (diluted 1 in 5 with distilled water) (5 mins.) (→ purple); Mann's (→ blue);
Or by method below :

- 1° *St.* Carazzi's hæmatoxylin.
- 2° "Blue" in tap-water.
- 3° Rinse in distilled water.
- 4° *St.* Mayer's mucicarmine. (20 mins.).
- 5° Wash in distilled water.
- [6° Counterstain in metanil yellow. ($\frac{1}{2}$ min.).]
- [7° Rinse in distilled water.]
- 8° Dehydrate. (Quickly).
- 9° Clear in xylene.
- 10° *Mt.* Canada balsam.

Omit stages 6° and 7° if desired.

Mucor.

- (a) *Temp. Mt.* Dip a portion rapidly in alcohol (70%) and mount in dilute glycerine.
- (b) *Hyphae. Temp. St.*
 - (α) *Protoplasm.* Iodine and basic fuchsin.
 - (β) *Cell Wall.* Schulze's soln. (→ violet).
- (c) *Perm.* As "Fungi" (iv) (a) (p. 156).

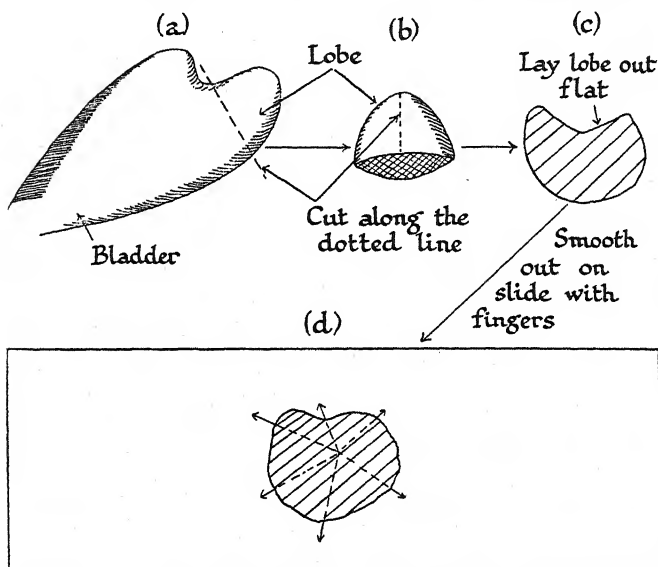
Muscle.

- (i) *Fix.* Formol-saline (5%).
- (ii) Do not impregnate with paraffin for longer than 1 hour or the tissue will be hardened.
- (iii) *St.* Hæmatoxylin and eosin Y (→ pink); van Gieson's (with or without iron hæmatoxylin (→ yellow); Mallory's (→ red); borax carmine; acetic acid (1%) (makes nuclei clear and transverse striations indistinct); picric acid (may be preceded by hæmatoxylin); Heidenhain's iron hæmatoxylin.

* After Carleton, *Histological Technique*, Oxford Medical Publications.

(iv) *Unstriated muscle in bladder of frog.**

- 1° Cut off a lobe of fresh frog's bladder. [Fig. 20 (a).]
- 2° Slit it up one side. [Fig. 20 (b).]
- 3° Spread it out flat. [Fig. 20 (c).]
- 4° Transfer to a slide with inner side uppermost.



[J. Carpenter.

FIG. 20.—Method of treatment of frog's bladder for preparation of unstriated muscle.

- 5° Spread out from centre with finger-tips, in all directions. (This removes epithelial cells which may hide the muscles.) [Fig. 20 (d).]
- 6° Stain in hæmatoxylin and eosin Y (aq.)
(Cytoplasm → pink; nuclei → blue).
- 7° Dehydrate.
- 8° Clear in xylene.
- 9° Mount in Canada balsam.

* With acknowledgments to J. G. Hawkes.

Mushroom. See "Agaricus" (p. 112).

Myelin Sheaths.

St. Mallory's (→ yellow to orange).

Mytilus. See "Anodon" (p. 127).

Nail.

(i) See "Skin" (p. 209).

(ii) *St.** Hæmatoxylin and eosin Y (→ bright pink);
iron hæmatoxylin and van Gieson's (→ black);
Mallory's (→ bright red).

Nematodes.

(i) *Rapid Examination.*

1° Kill in alcohol (90%).

2° Alcohol (100%).

(30 mins.).

3° Clear in "white" creosote.

(ii) *Unstained Preparations.*

(a) 1° Wash in sodium chloride (1% aq.).

2° Fix in boiling alcohol (70%). (On water-bath.)

3° Store in fresh alcohol (70%).

4° Place in a tube containing the following mixture:

Alcohol (100%) . . . 70 c.c.

Glycerine (5%) . . . 30 c.c.

(In an oven at 60° C. (24-48 hours) till alcohol
and water have evaporated.)

5° Examine in pure glycerine or glycerine-jelly.

(b) Mount in a mixture of:

Gelatin	20 parts	} Melt at moment of using.
Glycerine	100 "	
Water	200 "	
Phenol	2 "	

(iii) To roughen cuticle before staining: Potassium
hydroxide (5%). (2-4 hours).

* After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

(iv) *Stained preparations.**

Note: Nematodes are difficult to stain.

1° Either,

Kill and fix in a hot mixture of:

Mercuric chloride (satd. aq.) . 100 c.c.

Alcohol (95%) . . . 100 c.c.

Acetic acid (glacial) . . . 1 c.c.

or,

Treat for 24 *hours* or longer with eau de Labarraque (sodium hypochlorite), commercial soln. diluted with 4-6 vols. water, or used undiluted and hot for a short time.

2° *St.* Picric acid or borax carmine.

(v) See "*Ascaris*" (p. 129) and "*Proleptus*" (p. 202).

Nereis. Parapodia.

(i) *St.* Borax carmine and picric acid aq. or alc.

(ii) *Clear* in clove oil for a long time.

Nerve Collar, Helix. See "*Helix*" (p. 167).

Nerve Tissue.

(i) *Note:* Nerve tissue varies to such a degree with different locations that it is impossible here to give detailed methods. For these refer to a larger work on animal histology. The following must be regarded as very bare suggestions:

(ii) *Fix.* Formaldehyde (10%); Müller's fluid (*Daily changes for several weeks.*)

(iii) *St.* (a) *General work.* Methylene blue; van Gieson's after picric acid fixation.

(b) *Axis cylinders.*† Hæmatoxylin and eosin Y (→ mauve); iron hæmatoxylin and van Gieson's (→ grey); Mallory's (→ red).

(c) *Neuroglia fibres.*† Hæmatoxylin and eosin Y (→ pink to mauve); iron hæmatoxylin

* After Lee, *Microtometist's Vade-Mecum*, Churchill.

† After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

and van Gieson's (→ yellow); Mallory's (→ red).

(d) *Spinal cord*. Borax carmine; hæmatoxylin.

(e) *During dissections*. Picric acid aq.

(iv) *Method for nuclei, axons, dendrons in spinal cord*.

1° Smear grey matter from fresh spinal cord on cover-slip.

2° Fix in alcohol (95%).

3° Stain in warm Nissl's methylene blue, or methylene blue (1% alc.).

4° Wash in alcohol (95%).

5° Wash in alcohol (100%). (2 mins.).

6° Clear in benzene-phenol.

7° Mt. Canada balsam.

(v) *To emphasize nerve tissue during a dissection*.

Flood the area with picric acid (satd. aq.) and wash under tap.

Nitella.

St. Iodine and basic fuchsin.

Nuclei.

(i) *Fix*. Acetic acid (1%).

(ii) *St*.

(1) *Animal*.

(a) *Temp*.

Acetic acid (1%), or iodine (in unfixed tissue). Specially useful for squamous epithelium from cheek.

(b) *Perm*.

Hæmatoxylin and eosin Y (→ blue); iron hæmatoxylin and van Gieson's (→ black); Mallory's (→ red); Mayer's acid hæmalum; aceto-carmine aq. (stains and fixes; differentiate in water); Heidenhain's hæmatoxylin (→ dark blue); Leishman's (→ red); Loeffler's methylene blue.

(2) *Plant.**Perm.*

Hofmann's W.S. violet (0.002%); methyl green; safranin O; crystal violet and Gram's iodine, counterstained with orange G in clove oil; borax carmine; Hanstein's rosaniline-violet (\rightarrow red); Renaut's eosin Y-hæmatoxylin.

(3) *Animal and Plant.*(a) *Temp.*

Iron-aceto carmine.

(b) *Perm.*

Acetic-iodine green; Delafield's or Heidenhain's hæmatoxylin; eosin Y; safranin O; methylene blue.

Nucleoli.

St. Iron hæmatoxylin; eosin Y and methylene blue.

Obelia.(i) *Hydroid.*

(a) *Narcotize.* Menthol.

(b) *Fix.* Mercuric chloride (satd. aq.); formaldehyde (5%); alcohol (70%).

(c) *Stain.* Nigrosin water soluble (peculiarly superior to any other stain); Hanstein's rosaniline-violet.

(ii) *Medusa.*

Note: This provides a good test for the mastery of staining and differentiating.

(a) Avoid folding and crushing. Pick up with a wide-mouthed pipette held vertically.

(b) *Fix.* Mercuric chloride (satd. aq.); formaldehyde (5%); alcohol (70%).

(c) *Stain.* Borax carmine.

(d) *Clear.*

1° Place dehydrated specimen(s) in alcohol (100%) in a tube fitted with a cork. Add a few drops of cedar-wood oil,

carefully, down the side of the tilted tube. Cork tube and leave. (1 hour).

2° When the specimen has sunk to the bottom of the cedar-wood oil, pipette off alcohol and transfer specimen to fresh cedar-wood oil. ($\frac{1}{2}$ hour).

(e) *Mount.*

Place cleared specimen in some half-dried balsam on a slide. Add a little more balsam on top of specimen. Allow to half dry, add more balsam and cover.

(f) *To show otocysts.*

Overstain heavily and differentiate.

Note: Otoliths destroyed by acid used in differentiation.

Oedogonium.

(i) Fix in formo-acetic-alcohol.

(ii) See "Algæ" (p. 112).

Onion Root Tips. See "Mitotic Figures" (p. 177).

Opalina.

(i) *General method.* See "Protozoa" (p. 202).

(ii) *St.* Borax carmine; safranin O.

Ova. See "Amphibian Eggs" (p. 115), "Rana" (iii) (p. 205), and "Echinus" (ii) (p. 149).

Ovary.

(i) *Fix.** Bouin's fluid; formol-saline (4%); "corrosive-formaldehyde."

(ii) *St.** Hæmatoxylin and eosin Y; Mallory's (after "corrosive-formaldehyde").

(iii) *Clear.* Benzene-phenol or cedar-wood oil.

(iv) *Of Lumbricus.* See "Lumbricus" (iv) (p. 174).

* After Carleton, *Histological Technique*, Oxford Medical Publications,

Oxyntic Cells of Stomach Lining.

*St.** Hæmatoxylin and eosin Y (→ bright pink); iron hæmatoxylin and van Gieson's (→ yellow); Mallory's (→ orange).

Pancreas. See "Salivary Gland" (p. 207).

Paramecium.

- (i) *General Method.* See "Protozoa" (2) (iv) (p. 203).
- (ii) *St.* Methyl green; aceto-carmine; borax carmine; iron hæmatoxylin; light green S.F. yellowish.
- (iii) *To see food vacuoles.* Irrigate on slide with Indian ink or with very dilute neutral red.
- (iv) *To show vacuole movement.* Cover with a small piece of cover-slip and allow to become almost dry.
- (v) *To discharge trichocysts.* Tannic acid (1% aq.), or iodine (very dilute). (1 min. under cover-slip).

Parapodia, Nereis. See "Nereis" (p. 186).

Parasites in Blood.

- (i) *St.** Leishman's (→ blue with ruby red chromatin).
- (ii) See "Blood" (iv) (c), (d), and (e), (pp. 135, 136).
- (iii) *St.* Giemsa's (as Leishman's).

Patella.

Kill and fix. Formaldehyde (10%).

Pectic compounds (Mucilage; gums).

- (i) *St.* Ruthenium red.
- (ii) *Mt.* Glycerine; glycerine jelly; Canada balsam.

Penicillium.

- (a) *Temp. Mt.* Dip a portion rapidly in alcohol (70%) and mount in dilute glycerine.
- (b) *Perm.* As "Fungi" (iv) (a) (p. 156).

* After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

Pennatula.

- (i) *Kill.* Alcohol (70%).
- (ii) *Fix.* Formaldehyde (5%).

Periplaneta.

- (i) *To kill.*

Place in closed vessel of chloroform or drop into boiling water.

- (ii) *Mouth Parts.*

(a) *To remove flesh.* Boil in dilute sodium hydroxide (2%). (Not more than 5 mins.)

(b) *St.* As well unstained; but if it is desired to see the muscle tissue, do not boil in sodium hydroxide, but stain in picro-carmine.

- (iii) *Trachea.*

St. Unstained; borax carmine followed by picric acid (alc. or aq.) or methylene blue; picro-carmine.

Mt. Glycerine jelly or Canada balsam.

- (iv) *Salivary gland.*

1° Remove on to cover-slip below water to prevent collapse.

2° Fix alcohol (70%).

3° *St.* borax carmine; Delafield's hæmatoxylin.

4° Dehydrate thoroughly.

5° *Mt.* Canada balsam.

- (v) *Fat body.*

St. Borax carmine.

- (vi) *Leg muscle.*

St. van Gieson's; borax carmine.

Peronosporaceæ.

- (1) *Fix.* Alcohol (70%) and wash in water; or chrom-acetic, and wash in running water. (6-12 hours).

- (2) *Free-hand sections.*

(a) 1° Wash sections of herbaceous material in distilled water.

2° Stain in carbol-thionin. (5-8 mins.).

3° Wash in distilled water.

4° Dehydrate in alcohol (95%). (2 mins.).

5° Differentiate in orange G. (5 mins.).

[Saturated solution of orange G in alcohol (100%)].

6° Wash thoroughly in alcohol (100%).

7° Clear in xylene.

8° Mount in Canada balsam.

[Parasitic fungus (→ violet-purple); cellulose walls (→ yellow or green); lignified tissue (→ blue).]

N.B. By taking out of orange G, back to water the parasite is shown against unstained walls.

(b) See "Plant Tissue" (vii) II (a) (p. 195).

(3) *Wax-embedded sections.*

See "Fungi" (iv) (b) (p. 157). [Staining with safranin O and light green S.F. yellowish, as method (iv) (b) (1) (p. 159)]; and "Rusts" (p. 207).

Phloem.

St. (a) *Temp.* Corallin. (Callose → deep red; remainder → pale reddish yellow); Safranin O (especially for sieve plates).

(b) *Perm.* (i) *General.* Methyl green (→ blue).

(ii) *Bast fibres.* Hanstein's rosaniline violet (→ deep red).

(iii) *Bast parenchyma.* Hanstein's rosaniline violet (→ hardly stained).

(iv) *Callose.* Aniline blue W.S. (dissolves from cell walls in glycerine).

(v) *Sieve Plates.* Aniline blue W.S.; Delafield's hæmatoxylin; Hoffmann's blue.

(vi) *Sieve Tubes.* Hanstein's rosaniline violet (→ hardly stained); eosin Y (alc.).

Pituitary.*

- (i) *Fix.* Formol-saline (5%); "corrosive-formaldehyde."
- (ii) *St.* Hæmatoxylin and eosin Y; eosin Y and Borrel's methylene blue (p. 230); Mallory's (after "corrosive-formaldehyde").

Planaria and similar Animals.

- (i) *Fix.*
 - 1° Place living animal on a slide.
 - 2° Drop a little Bouin's fluid on its "tail." The animal crawls along the slide.
 - 3° Gradually add more Bouin's. This method fixes the animal stretched out. If dropped direct into Bouin's it will curl up.
- (ii) *St.* etc.
 - See "Fasciola" (p. 152).
- (iii) *To keep the animal flat.*
 - During the processes, place it between two slides and tie round with cotton.
- (iv) See "Fasciola" (p. 152).

Plankton.

- (i) When examining, raise the cover-slip on two streaks of vaseline. Ring the slip with vaseline if the preparation is temporary.
- (ii) When processing (usual methods), treat quantities in a tube as recommended in Method P-3 (p. 83).
- (iii) *Kill and fix.* Formaldehyde (3%).
- (iv) *St.* Borax carmine.
- (v) See "Aquatic and Marine Organisms" (p. 128).

Plant Tissue.

- (i) *Fixing fresh sections.*
 - See "Plant Tissue" (a) (p. 22).
- (ii) *Fix.*
 - (a) Chrom-acetic.

* After Carleton, *Histological Technique*, Oxford Medical Publications.

- (b) Alcohol (70%) 85 c.c.
 Formaldehyde (40%) 10 c.c.
 Acetic acid (glacial) 5 c.c.
- (c) Formalin-alcohol (for delicate algæ and fungi).
- (iii) *Preserve.*
 See "Plant Tissue" (p. 318).
- (iv) *Cutting fixed tissue without embedding.*
 First stiffen tissue in alcohol (100%) (15 mins.),
 and cut in alcohol (50%).
- (v) *St.*
 (a) *Bulk.* Borax carmine.
 (b) *Fresh Sections.* Safranin O and hæmatoxylin
 (lignified tissue → red; cellulose → purple);
 safranin O and light green S.F. yellowish in
 clove oil (lignified tissue → red; cellulose →
 green); Hanstein's rosaniline violet (esp.
 monocot. stem).
 (c) *Protein cell contents.*
Temp. Iodine (→ brown).
 (d) *Protoplasm.* Eosin Y; erythrosin bluish.
 (e) *Thick objects and whole mounts.* Hæmatoxylin
 (differentiated).
- (vi) *Mt.*
 (a) See p. 44.
 (b) Water-washed, stained, sections may be first
 soaked and then mounted in Farrants' medium.
 (No dehydrating, clearing, or ringing required.)
 (c) Canada balsam.
- (vii) See also "Bundle Ends" (p. 138), "Epidermis"
 (p. 150), "Lignified Tissue" (p. 172), "Lignin"
 (p. 173), "Mesophyll" (p. 177), "Mitotic Figures"
 (p. 177), "Phloem" (p. 192), "Stomata" (p. 212),
 and "Xylem" (p. 217).
- (viii) *General Histology.*
 N.B. Before starting any method, consult Chapter
 VII (p. 220) to find out all you can about the stain, its
 uses, and how it is made up. Those methods most suit-

able for a beginner are indicated by a heavy line at the left-hand side. To avoid repetition, the final processes of dehydration, clearing, and mounting have not been listed unless special methods are desirable. In any case, details of the processes will vary according to the dehydrating agent (ethyl alcohol, cellosolve, dioxan, butyl alcohol) in use, and reference should be made to the appropriate section on dehydration (p. 33). Canada balsam is a satisfactory permanent mountant.

Full details of routine methods are given in Chapter IV (p. 71), and Chapter V (p. 88).

I. *Single Staining.*

(a) DELAFIELD'S HÆMATOXYLIN (p. 240).

A general purpose stain for sections.

1° Stain in stock solution of Delafield's hæmatoxylin. (1 min.).

2° Wash in tap-water or alkaline water (p. 285). (2 mins.).

(b) BORAX CARMINE (p. 230).

Useful for bulk staining and especially for *Fucus*.

1° Stain in stock solution of borax carmine. (24 hours).

2° Differentiate in acid alcohol. (15 mins.).

3° Transfer to alcohol (70%). (1 hour).

4° Transfer to alcohol (100%). (1 hour).

II. *Counterstaining.*

(a) SAFRANIN O (p. 280) COUNTERSTAINED WITH LIGHT GREEN S.F. YELLOWISH (IN CLOVE OIL) (p. 260).

Either, 1° Stain in stock solution of safranin O.

(5 mins.).

2° Dehydrate from alcohol (50%) to alcohol (100%).

3° Counterstain in light green S.F. yellowish (in clove oil). (2 mins.).

4° Wash in alcohol 100%.

($\frac{1}{2}$ min. in a covered vessel).

5° Clear in clove oil.

6° Pass quickly through xylene.

7° *Mt. Canada* balsam.

Or, 1° Alcohol (50%).

2° Safranin O.

3° Dehydrate alcohol (70%), (90%).

4° Light green S.F. yellowish (in alcohol and clove oil).

5° Clear in clove oil/alcohol (90%) : : 60/40.

(The addition of 1 drop of dilute acid to the watch-glass of clove oil/alcohol will differentiate.)

6° Pass through a watch-glass of benzene-phenol to which 1 drop of alcohol (100%) has been added.

7° *Mt. Canada* balsam.

Or, *1° Cellosolve.

(2 mins.).

2° Safranin O.

(Time varies with tissue).

3° Cellosolve. (1 min. to remove excess safranin O).

4° Light green S.F. yellowish in clove oil.

(2 mins.).

5° Clove oil.

(To remove excess stain).

6° Pass quickly through xylene.

7° *Mt. Canada* balsam.

(For detailed results of staining by this method, see Method P-5 (p. 86). In general: lignin → red; cellulose → green.)

(b) SAFRANIN O (p. 280) COUNTERSTAINED WITH DELAFIELD'S HÆMATOXYLIN (p. 240).

1° Stain in stock solution of safranin O.

(1 min.).

2° Transfer to alcohol (50%).

(2 mins.).

* From information supplied by Messrs. T. Gerrard & Co. from whom "Cellosolve" may be obtained.

- 3° Wash distilled water. (1 min.).
- 4° Stain in stock solution of Delafield's hæmatoxylin. (3 mins.).
- 5° Transfer to *tap*- or alkaline water. (1 min.).
(Sections to look faintly purple.)

(Lignin → red; cellulose → blue).

- (c) SAFRANIN O (p. 280) AND ANILINE BLUE W.S. (ALC.) (p. 226), or PICRIC-ANILINE BLUE W.S. (p. 272).

- 1° Safranin O. (5 mins.).
- 2° Wash alcohol (85%).
- 3° Aniline blue W.S. (alc.), or picric-aniline blue W.S. (2 mins.).
- 4° Differentiate in acid alcohol. (*Few seconds*).
- 5° Wash alcohol (95%). (1 min.).

(Lignin → red; cellulose → blue).

- (d) CYANIN (p. 239) AND BISMARCK BROWN Y Aq. (p. 230).

- 1° Stain in stock solution of cyanin. (10-12 mins.).
- 2° Wash in distilled water.
- 3° Stain in stock solution of Bismarck brown Y (aq.). (15-20 mins.).
- 4° Wash in distilled water.

(Lignin → blue; cellulose → brown).

- (e) MALACHITE GREEN (p. 262), OR IODINE GREEN (p. 256), AND MAGDALA RED (p. 262).

- 1° Malachite green (aq.), or iodine green (aq.). (1 min.).
- 2° Wash out some green with alcohol (95%).
- 3° Wash in distilled water.
- 4° Magdala red (aq.). (10 mins.).
- 5° Wash in distilled water.

(Lignin → green; cellulose → pink).

- (f) ACID FUCHSIN (p. 222), AND MALACHITE GREEN (p. 262), OR IODINE GREEN (p. 256).

1° Acid fuchsin (aq.). (2 mins.).

2° Wash in distilled water.

3° Malachite green (aq.).

(1 min.—until fuchsin is displaced from cellulose).

4° Wash in distilled water.

(Lignin → green ; cellulose → red).

- (g) ANILINE CRYSTAL VIOLET (p. 227), GRAM'S IODINE (p. 251), AND BISMARCK BROWN Y (ALC.) (p. 230).

1° Crystal violet (aq.) with aniline oil.

(5 mins.).

2° Drain off excess.

3° Gram's iodine.

(3 mins.).

4° Wash alcohol (95%).

5° Bismarck brown Y (alc.).

(5 mins.).

(Lignin → violet ; cellulose → brown).

- (h) CRYSTAL VIOLET (p. 238), GRAM'S IODINE (p. 251), AND EOSIN Y (IN CLOVE OIL) (p. 245).

1° Wash sections in alcohol (70%).

2° Stain in stock solution of crystal violet (alc.).

(3–5 mins.).

3° Wash rapidly in alcohol (75%), or (95%).

4° Transfer to Gram's iodine. (1–2 mins.).

5° Wash thoroughly in alcohol (90%).

6° Dehydrate in alcohol (100%).

7° Stain and clear in eosin Y in clove oil.

8° Pass through fresh oil of cloves.

9° Wash in xylene.

(Quickly).

10° Mt. Canada balsam.

(Lignin → violet ; cellulose → red).

III. *Differential Staining with free Dye-acid.*

XYLENE-EOSIN Y (p. 288) OR XYLENE-ERYTHROSIN BLUISH (p. 289).

1° Stain in Hofmann's W.S. violet (1%)—if desired—and, if necessary, differentiate in acid alcohol.]

2° Dehydrate sections either, alcohol (30%), (50%), (70%), (90%), (2 mins. each);
or, cellosolve.

(1 min.).

3° Wash alcohol (100%). [*To remove stain from cellulose walls.*] (Unnecessary if cellosolve used as dehydrating agent.)

4° Transfer to xylene.

5° Stain in xylene-eosin Y (or xylene-erythrosin bluish). (*Colour develops immediately. Prolonged staining no advantage.*)

6° *Mt. Canada* balsam in xylene.

[Results with xylene-erythrosin bluish; cellulose → red; cytoplasm → light red; nuclei → red; plastids → light red (not consistent); intercellular tryones → red; vessels in formation at inner cambial boundary → half red; protoxylem vessels → half red; lignin → colourless; metaxylem → colourless; sclerenchyma → colourless; cork → colourless; pith cells → colourless.]

[Results with xylene-eosin Y; as above, but a more orange-red. The writer has not found this method so satisfactory.]

IV. *Differential Substitution Staining with Free Dye-base and Free Dye-acid.*

XYLENE-METHYLENE BLUE (p. 289), (or xylene-nile blue sulphate) AND XYLENE-ERYTHROSIN BLUISH (p. 289), (or xylene-eosin Y).

1° Dehydrate sections in alcohol (30%), (50%), (70%), (90%), (100%), (*Few seconds each*);
or in cellosolve. (1 min.).

- 2° Transfer to xylene. (*Few seconds*).
- 3° Transfer to xylene-methylene blue (or xylene-nile blue sulphate). (*Few seconds*).
- 4° Transfer to xylene-erythrosin bluish (or xylene-eosin Y). (*Few seconds*).
- 5° *Mt. Canada* balsam in xylene.

[Results with xylene-methylene blue and xylene-erythrosin bluish; cellulose → red; cortex → purplish; cytoplasm → light red; nuclei → red; plastids → light red (not consistent); inter-cellular tryones → red; cambium → red; vessels in formation at inner cambial boundary → half red; protoxylem vessels → half red; lignin → blue; metaxylem → blue; sclerenchyma → blue; cork → colourless; pith cells → colourless.]

Platyhelminthes. See "*Fasciola*" (p. 152), "*Planaria*" (p. 193). and "*Tænia*" (p. 213).

Pleurobrachia.

- (a)* 1° Kill in formaldehyde (5% in sea-water).
- 2° Fix in formaldehyde (5%).
- 3° Store in alcohol (70%).
- (b) *St.* Methyl green.

Pollen Grains.

- (i) 1° Tease out stamens in water on slide.
- 2° Drain off water and leave pollen on slide.
- 3° Add 1 drop glycerine (for temp. mount) or glycerine jelly (for perm. mount).
- 4° Mix pollen into glycerine (jelly) with needle.
- 5° Cover.
- (ii) *Clear* † Phenol (aq.); chloral hydrate.
- (iii) See also "*Protozoa*" (2) (iv) (p. 203).
- (iv) *To grow tubes.* See "*Hanging-Drop Cultures*" (p. 302).

* After Bolles-Lee, *Microtomist's Vade-Mecum*, Churchill.

† Recommended by Strasburger, *Handbook of Practical Botany* (English edn.). Allen and Unwin.

Pollen Tubes.

- (i) *To grow.* See "Hanging-Drop Cultures" (p. 302).
- (ii) *St.* Acetic-methyl green.

Polystomella.

The following method is a good test for the mastery of staining and differentiating technique :

- 1° Dehydrate in alcohol (30%), (50%), (70%).
(5 mins. each).
- 2° Decalcify by placing in acid alcohol.
(Till shell removed).
- 3° Wash in alcohol (70%).
(Till all acid alcohol removed : test with litmus paper).
- 4° Stain in borax carmine. (Overstain deeply).
- 5° Differentiate in acid alcohol.
- 6° Dehydrate in alcohol (70%), (90%), (100%).
(2 mins. each).
- 7° Clear in benzene-phenol.
- 8° *Mt.* Canada balsam.

Polytoma.

To see flagella.

Irrigate under cover-slip with dilute iodine.

Polyzoa.

- (i) *To kill.* Hot mixture of mercuric chloride (sat. aq.) and iodine (alc. soln.).
- (ii) *Fix.* Alcohol (70%).
- (iii) See "Aquatic and Marine Organisms" (p. 128).

Porifera.

- (i) *To kill and fix.*
 - (a) Alcohol (70%).
 - (b) See "Aquatic and Marine Organisms" (p. 128).
- (ii) *Spicules.*
 - 1° Boil small portion of sponge in conc. sodium hydroxide (aq.) till matrix dissolves.

- 2° Allow spicules to settle.
- 3° Decant sodium hydroxide.
- 4° Wash by decantation.
- 5° Dehydrate by decantation.
- 6° Clear in benzene-phenol.
- 7° *Mt.* Canada balsam.

Proleptus.

This nematode is often found in the dogfish (*Scyllium* and *Acanthias*).

(i) See "Nematodes" (p. 185).

(ii)* 1° Place specimen in

Alcohol (100%)	22 c.c.
Chloroform	15 c.c.
Acetic acid (glacial).	5 c.c.
Phenol. Add crystals to increase volume by		10 c.c.

2° Add oil of wintergreen drop by drop.

3° Add Canada balsam slowly.

4° *Mt.* Canada balsam.

Protococcus.

(i) *General Method.* See "Protozoa" (below).

(ii) *St.* Iodine and basic fuchsin.

Protoplasm (especially plant protoplasm).

St. (a) *Temp.*

Iodine (→ brown).

(b) *Perm.*

Alkannin (→ pale rose-red) ($\frac{1}{2}$ hour); eosin Y;
erythrosin bluish; Hanstein's rosaniline violet
(→ bluish violet); Hoffmann's blue.

Protozoa.

(1) *Temporary Preparations.*

(i) Place a drop of the fluid containing the protozoa on a slide, cover, and treat throughout by irrigation.

* After Green, *School Science Review*, XV; Dec. 1933.

(ii) *Alive.*(a) *To prevent movement under the slide.*

(α) Mount in a drop of carragheen solution (p. 234).

(β) Place a few strands of cotton wool on the slide.

(b) *To show cilia.*

Add a drop of glycerine or Indian ink or carmine suspension.

(c) *To slow up vacuole movement.*

Add sodium chloride (0.25% aq.).

(d) *St.* Very dil. methylene blue aq.; Nile blue sulphate; very dilute neutral red.

(e) See Method T-1 (p. 74).

(iii) *Dead.*

(a) *Fix.* Osmium tetroxide (0.25%) in drop on slide under cover-slip; osmium tetroxide vapour; glacial acetic acid vapour.

(b) *St.* Acetic-methylene blue (kills and stains); safranin O (2-3 mins.); Hofmann's W.S. violet (2-3 mins.); hæmatoxylin; methyl green; aceto-carmine; borax carmine.

(c) See Method T-2 (p. 75).

(2) *Permanent Preparations.*

(i) *Fix.* "Corrosive-acetic" (Poison—care) (Wash 3-4 times with water, or, if an alcoholic stain is to be used, with alcohol. Add a few drops of iodine solution to the washing liquid. If colour disappears add more iodine); osmium tetroxide (0.25%); Schaudinn's fluid. (Wash as "corrosive acetic.")

(ii) *St.* As temp. preparations.

(iii) *Large Specimens.* Treat as ordinary material, carrying out the operations with a quantity of animals in a piece of glass tubing as recommended in Method P-3 (p. 83).

(iv) *Small Specimens.*

1° Smear slide thinly with egg albumen or saliva.

- 2° Place drop of culture on slide.
 - 3° Air dry as long as possible but *not to kill animals*.
 - 4° Add "corrosive-acetic" with pipette. (2 mins.).
 - 5° Wash distilled water, to which a few drops of iodine have been added. Add more iodine as colour disappears.
 - 6° St. Delafield's hæmatoxylin. (20 mins.— $\frac{1}{2}$ hour).
 - 7° Differentiate in acid alcohol.
 - 8° Wash alcohol (70%). (1 min.).
 - 9° Wash alcohol (90%). (1 min.).
 - 10° "Blue" in alkaline alcohol.
 - 11° Dehydrate.
 - 12° Clear in benzene-phenol.
 - 13° Mt. Canada balsam.
- (v) *Shelled Protozoa*. See "Polystomella" (p. 201).

Pyronema.

Oogonia. See "Fungi" (iv) (d) (p. 164).

Rana.

(i) *To pith*.

Hold the frog in a duster. Chloroform it by holding a pad soaked in chloroform over its nostrils. Extend its hind limbs and feel with the nail for the depression beneath the skin at the back of the head (articulation of skull and vertebral column)—it lies in a line joining posterior borders of the two tympanic membranes. Divide the skin and muscles here to expose the neural canal. Pass a stout wire (a blunt curved mounted needle is useful) into the cranium, only partially withdraw the probe (to lessen risk of missing the neural canal), turn it round and pass it down the neural canal in the vertebral column. If chloroform is not available, the frog may be rendered unconscious by giving it a sharp jab with a blunt scalpel in the depression between atlas and skull.

(ii) *To kill*.

Allow at least 20 mins. in a closed vessel containing a pad of cotton wool soaked in chloroform.

(iii) *Spawn.*(a) *Gatenby's Method.**

1° Place eggs in Gatenby's fluid.

(Use at least 40 c.c. for 20-30 eggs.)

(24 hours).

2° Shake off albuminous envelope.

3° Wash in running water. (1 hour).

4° Dehydrate in alcohol (30%), (50%), (70%).

(30 mins. each).

5° Dehydrate in alcohol (90%). (1 hour).

6° Dehydrate in alcohol (100%) (two changes).

(1 hour each).

7° Clear in benzene. (15 mins.).

8° Add shreds of paraffin wax.

(In oven: do not overheat.) (30 mins.).

9° Transfer to pure wax. (15-20 mins.).

10° Transfer to fresh wax. (15-20 mins.).

11° Embed.

12° Cut at 6μ with a very sharp razor.

13° Usual processes leading to staining (any method) and finishing.

(b) *To preserve.* See p. 316.(iv) *Tadpole.**Fix.* Bouin's fluid. (Small, 12 hours; large, 18 hours.)

Wash in several changes of alcohol (70%).

(v) *Unstriated muscle fibres in bladder.* See "Muscle"

(iv) (p. 184).

Resin.*St. Perm.* Alkannin (\rightarrow deep red) (1 hour); Hanstein's rosaniline violet (\rightarrow blue).**Retina.** See "Eye" (p. 152).**Rhabditis.**

Nematode parasitic in nephridium of earthworm.

(i) *Culture.* See "Rhabditis" (p. 310).* Adapted from Bolles-Lee, *Microtomist's Vade-Mecum*. Churchill.

(ii) *To show cell division after fertilization.*

1° Obtain female specimens as explained under notes on culture.

2° Transfer the specimen to a slide with a camel-hair brush, and burst it by gentle pressure with a cover-slip.

3° Examine with a $\frac{1}{8}$ " objective.*Note*: This preparation is a good test of the efficiency of a micro-projector for screen demonstration to a class.**Root.**

(i) See "Plant Tissue" (p. 193).

(ii) See "Mitotic Figures" (p. 177).

Rotifers (Rousselet's method).1° Narcotize. (*About 15 mins. till cilia cease moving*).

Purpose is to keep cilia extended. Run the narcotic gently down the side of the containing vessel.

Suitable narcotics:

(a) Cocaine hydrochloride (1%).

(b) Cocaine hydrochloride (2%) 30 c.c.

Alcohol (96%) 10 c.c.

Distilled water 60 c.c.

2° Kill and fix.

Suitable agents:

(a) Weak Flemming's fluid: (3-4 mins.).

Chromic "acid" (1%) 75 c.c.

Osmium tetroxide (2%) 20 c.c.

Acetic acid (glacial) 5 c.c.

(b) Osmium tetroxide (0.25%). (1 min.).

If blackened, bleach in hydrogen peroxide.

3° Wash in distilled water. (*Several times*).4° Stain in *very weak* Flemming's fluid viz.:

Weak Flemming's [as 2° (a) above] 1.5 c.c.

Distilled water 98.5 c.c.

(Watch under the microscope—the nervous system should become slightly tinged with yellow).

- 5° Mount in formalin (3%) in a cavity slide.
(Transfer the animals with a dipping pipette).
6° Blot off any moisture round the edges of the cavity.
7° Cover gently.
8° Ring with gold size.

Rusts.

- (i) 1° Fix in acetic-alcohol. (15-30 mins.).
2° Wash in alcohol (90%) or (95%). (2-3 hours).
3° Either,
(α) For future use, store in Calberla's fluid.
or (β) For hand-sections, refer to "Fungi" (iv) (c) (p. 162).
or (γ) For wax-embedded sections dehydrate in alcohol (100%), and proceed from process 12° of "Fungi" (iv) (b) (p. 158), staining by method (iv) (b) (1) (p. 159), or with Delafield's hæmatoxylin counterstained with Congo red. Preparations stained with Congo red must be well washed before mounting in Canada balsam.*
(ii) See also "Peronosporaceæ" (p. 191).

Salivary Gland.

- (i) *Fix.* "Corrosive-formaldehyde"; formol-saline.
(ii) *St.* Hæmatoxylin and eosin Y; Mallory's (after "corrosive-formaldehyde").
(iii) *Mucin.* See "Mucin" (p. 183).
(iv) *Of Periplaneta.* See "Periplaneta" (p. 191).

Sea Anemone. See "Anemone" (p. 116).

Sea Fir. See "Sertularia" (p. 208).

Sea Mat. See "Polyzoa" (p. 201).

Sea Mouse. See "Aphrodite" (p. 128).

* Recommended by Gwynne-Vaughan and Barnes, *Structure and Development of the Fungi*, Cambridge University Press.

Sea Pen. See "Pennatula" (p. 191).

Sea Slug. See "Doris" (p. 149).

Sea Squirt. See "Tunicate" (p. 216).

Sea Urchin. See "Echinus" (p. 149).

Seaweeds (Small).

(i) See "Algæ" (p. 112).

(ii) Proceed as for "Echinus" (ii) (p. 149) from process 2° onwards.

Sections, Frozen. Refer to more advanced books suggested in Bibliography (p. 323).

Seeds, To Mount.

(After A. Powell Jones, *Science Masters' Book*, Part II, Murray, London.)

1° Cut pieces of 4-ply wood 3" \times 1 $\frac{3}{8}$ ".

2° Bore a hole $\frac{7}{8}$ " diameter in the centre of each piece. (Two holes, suitably spaced, may be bored if desired, but difficulty may be found in retaining the "slide" on the microscope stage.)

3° Glue a clean slide on to one side of the wood. Avoid getting glue on the glass over the hole(s).

4° Place the seeds in the cavity thus made. (Loose, or fixed with a touch of Canada balsam.)

5° Glue another slide on the wood to cover the cavity, observing the same precautions as before.

6° Label, either in the usual position, or, if two holes are used, by means of a piece of paper placed, after suitable inscription, on the strip of wood between the two holes which are then covered as in 5°. If the latter method is adopted, avoid glueing the label.

Sertularia.

(i) *Kill.* In a hot mixture of mercuric chloride (sat. aq.) and iodine (alc. soln.).

(ii) *Fix.* Alcohol (70%).

Skeleton.

To stain the whole skeleton of small vertebrates without staining the flesh, see "Bone" (iii) (p. 137); and "Cartilage" (iii) (p. 140).

Skin.

- (i) See also "Epithelia" (p. 151).
- (ii) *Method for sections*.*
 - 1° Fix formol-saline (5%). (24 hours).
 - 2° Dehydrate alcohol (100%). (24 hours).
 - 3° Clear acetone. (1 hour).
 - 4° Impregnate paraffin wax (m.pt. 50° C.). (6 hours).
 - 5° Section at 2-4 μ .
 - 6° St. Heidenhain's iron hæmatoxylin and van Gieson's.
- (iii) *Stratum lucidum*.†
 - St. Hæmatoxylin and eosin Y (\rightarrow bright pink);
 - iron hæmatoxylin and van Gieson's (\rightarrow yellow);
 - Mallory's (\rightarrow orange).
- (iv) *Of inside of cheek*. See "Epithelia" (v) (p. 151); and Methods T-4 (p. 77); T-5 (p. 78); P-2 (p. 82); and P-4 (p. 85).
- (v) *Keratin in*. See "Keratin" (p. 171).

Slugs.

- (i) *Kill*. Drown in water.
- (ii) *Fix*. Alcohol (60%).

Spermatozoa.

- (i) *General method*.‡
 - 1° Smear a cover-slip with glycerin-egg albumen.
 - 2° Smear cut testis on the cover-slip.
 - 3° Fix Bouin's fluid. (3 mins.).

* After Carleton, *Histological Technique*, Oxford Medical Publications.

† After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

‡ After Lee, *Microtometist's Vade-Mecum*, Churchill.

- 4° Wash alcohol (50%). (2 mins.).
- 5° Dehydrate alcohol (70%). (2 mins.).
- 6° " " (90%). (Overnight).
- 7° Hydrate to distilled water.
- 8° St. crystal violet. (3 mins.).
- 9° Dehydrate.
- 10° Clear.
- 11° Mt. Canada balsam.
- (ii) St. Hofmann's W.S. violet (0.25%) (2-3 mins.);
safranin O.
- (iii) See "Protozoa" (p. 202).

Spermatozoids.

- (i) St. Crystal violet (nuclear).
- (ii) *To slacken movement.*
Gum arabic (10% aq. filtered through swansdown).

Spinal Cord. See "Nerve Tissue" (p. 186).

Spindle Fibres. See "Mitotic Figures" (p. 177).

Spirogyra.

- (i) See "Algæ" (p. 112).
- (ii) *Fix.* Formo-acetic-alcohol; Bouin's fluid.
- (iii) St. Aniline blue W.S.
- (iv) 1° Place algæ in glycerine (10%) in watch-glass.
- 2° Allow water to evaporate by heating in oven to 30° C.
- 3° When soln. has thickened almost to strength of pure glycerine, transfer to drop of pure glycerine on slide.
- 4° Cover.
- 5° Ring with Canada balsam or gold size.

Spleen.

- (i) See "Lymph Glands" (p. 176).
- (ii) *Impregnation with paraffin.* Not for longer than 1 hour, or tissue will harden.

(iii) *Smears.*

- 1° Press slide on freshly cut spleen. Pull slide off so that some spleen is sucked on to the slide.
- 2° Wave in the air to dry.
- 3° Treat as for remaining stages of blood films (iv), p. 134.

Sponge. See "Porifera" (p. 201).

Squamous Epithelium. See "Epithelia" (p. 151); and Methods T-4 (p. 77); T-5 (p. 78); P-2 (p. 82); and P-4 (p. 85).

Starch.

- (i) *Granules.* To see these, cut potato at *right-angles* to the skin, and mount sections in water.
- (ii) *St. etc.*
 - (a) *Temp.*
Chloral hydrate (→ starch grains swell); corallin (→ rose); iodine (→ blue); Schulze's soln. (→ blue).
 - (b) *Perm.*
Safranin O (→ pink).
- (iii) *Unstained mounts.*
 - 1° Add alcohol (90%) to a little dry starch in a small test-tube and shake up.
 - 2° Decant alcohol.
 - 3° Add "Euparal Essence" and shake up.
 - 4° Transfer a little of the suspension to a slide.
 - 5° Drain off "Euparal Essence."
 - 6° Mt. in "Euparal" which, having a lower refractive index than Canada balsam, enhances visibility.

Stem.

- (i) *St.* Hanstein's rosaniline violet.
- (ii) See "Plant Tissue" (p. 193); and Methods T-6 (p. 79); T-7 (p. 80); P-1 (p. 81); and P-5 (p. 86).

Stentor.

- (i) See "Protozoa" (p. 202).
- (ii) See "Volvox" (p. 216).

Stereum purpureum.

See "Fungi" (iv) (c) (p. 162), but stain with Delafield's hæmatoxylin.

Sting of Wasp or Bee.

Kill the insect [see "Insects" (i) (a), p. 170], compress its abdomen to extrude the poison sac and sting, and cut these away with a sharp scalpel.

Mt. (a) *Temp.*

Glycerine (10%).

(b) *Perm.*

Usual methods.

Stomach. See "Alimentary Canal" (p. 115).

Stomata in Leaf.

- (i) (a) Use material preserved in alcohol (70%).
- (b) *St.* Delafield's hæmatoxylin.
- (ii) See "Epidermis of Leaf" (i) (p. 150); and Methods T-1 (p. 74); and T-2 (p. 75); and "Plant Tissue" (a) (p. 22); (v) (b) and (viii) (pp. 194-8).

Stomata in Pavement Epithelium. See "Epithelia" (iv) (p. 151).

Suberin.

St. (a) *Temp.*

Schulze's soln. (→ yellow brown).

(b) *Perm.*

Alkannin (→ red) (slow); Sudan III.

Sunflower.

See "Helianthus" (p. 167).

Suprarenal Body.*

- (i) *Fix.* Immediately after death. Formaldehyde (5%); "corrosive-formaldehyde."
- (ii) *St.* Delafield's hæmatoxylin and eosin Y; Heidenhain's iron hæmatoxylin and van Gieson's.
- (iii) *To show adrenalin.*
 - 1° Fix potassium dichromate (2% aq.). (30 mins.).
 - 2° Add 10% of formaldehyde (40%) and continue fixation. (3 days).
 - 3° Wash in running water.
 - 4° Embed in paraffin wax.
 - 5° *St.* sections Delafield's hæmatoxylin (adrenalin → yellow-brown).

Tadpole. See "Rana" iv (p. 205).

Tænia. (Scolex, Proglottides, and Cysticercus.)

- (i) See "Fasciola" (p. 152), and "Planaria" (p. 193).
- (ii) *Fix.* Bouin's fluid; alcohol (70%); mercuric chloride (satd. aq.).
- (iii) *St.* (a) *Whole mounts.* Borax carmine (prolonged overstain and differentiate).
- (b) *Sections.* Delafield's hæmatoxylin or Mallory's.
- (iv) *Useful method.*
 - 1° Wash in sodium chloride (1% aq.).
 - 2° Fix in hot "corrosive-acetic" (50° C.). Allow to stand. (Till cool).
 - 3° Wash in running water. (12 hours).
 - 4° *St.* Borax carmine.
- (iv) *Cysticercus.*
 - (a) *To evert scolex.*
 - (i) Place in warm saline.
 - (ii) Collect cysticerci from mesentery and liver of rabbit. Place a cysticercus on the centre of a slide, cover with a piece of filter

* After Carleton, *Histological Technique*, Oxford Medical Publications.

paper. Place a piece of $\frac{1}{4}$ " diam. glass rod (about 8" long) transversely on the slide and, with the flat palm of the hand, roll the rod, with gentle pressure, over the filter paper. The scolex will be everted and the cysticercus fixed to the slide, while the paper absorbs the moisture.

(b) *St. Everted cysticercus*. Borax carmine.

Tannin.*

- (i) *St.* Hanstein's rosaniline violet (\rightarrow foxy-red).
 - (ii) 1° Fix small pieces of tissue in copper acetate (7% alc.). (8-10 days).
 - 2° Cut sections and place in ferrous sulphate (0.5% aq.). (3 mins.).
 - 3° Wash in water.
 - 4° Alcohol (70%).
 - 5° Mt. Glycerine jelly.
- Note:* Tissue fixed in copper acetate may be preserved in alcohol (70%) and cut for treatment with ferrous sulphate later.
- (Tannin regions \rightarrow dark iron-blue.)

Tape Worm. See "Tænia" (p. 213).

Teasing Small Animals. See "Animals, Small" (p. 116).

Teeth.

- (i) Treat as bone (p. 137).
- (ii) *St.*† (a) *Dentine*. Hæmatoxylin and eosin Y (\rightarrow pink); iron hæmatoxylin and van Gieson's (\rightarrow black); Mallory's (\rightarrow bright red).

* After Strasburger, *Handbook of Practical Botany* (English edn.). Allen and Unwin.

† After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

- (b) *Enamel*. Hæmatoxylin and eosin Y (→ pink); iron hæmatoxylin and van Gieson's (→ grey to black); Mallory's (→ orange).

Tendon. See "Ligament" (p. 172).

Testis.

- (i) See "Spermatozoa" (p. 209).
(ii) * See "Ovary" (p. 189), but do *not* fix mammalian testis in formol-saline.
(iii) *Harden*. Alcohol (70%).
(iv) *St.* (a) See "Animal Tissue—General histology" (p. 117).
(b) Ehrlich's hæmatoxylin and eosin Y (alc.).

Textile Fibres. See "Fibres" (p. 154).

Thymus.*

- (i) *Fix.* Formol-saline (5%); "corrosive-formaldehyde."
(ii) *St.* Heidenhain's iron hæmatoxylin and van Gieson's; Mallory's (after "corrosive-formaldehyde").

Thyroid.*

- (i) *Fix.* "Corrosive-formaldehyde"; formol-saline (5%).
(ii) *St.* Mallory's (after "corrosive-formaldehyde"); hæmatoxylin and eosin Y.

Trachea of Insect. See "Periplaneta" (p. 191) and "Acarine" (p. 111).

Tribonema.

- (i) See "Algæ" (p. 112).
(ii) *Fix.* Formo-acetic-alcohol.
(iii) *St.* Hæmatoxylin (mordant with liquor ferri).

* After Carleton, *Histological Technique*, Oxford Medical Publications.

Trypanosome.

- (i) See "Blood" (iv) (b), (c), (d) and (e) (p. 135).
- (ii) *St.* Hæmatoxylin; Leishman's.

Tubularia. See "Cœlenterates" (p. 144).

Tunicates.

Kill and fix. Alcohol (70%).

Ulothrix.

- (i) See "Algæ" (p. 112).
- (ii) 1° Fix and stain in nigrosin water soluble, dissolved in Bouin's. (3 months).
- 2° Wash in distilled water.
- 3° Place in glycerine (10%) and allow soln. to concentrate at room temperature.

Vegetable Fibres. See "Fibres" (p. 154).

Venation, to Distinguish in Leaves. See "Bundle Ends" (p. 138).

Volvox.

- (i) See "Protozoa" (p. 202).
- (ii) *St.* Methyl green.
- (iii) *Mt.* Formaldehyde (5%); glycerine, 1 part, with formaldehyde (5%), 1 part.
- (iv) *To mount, and preserve green colour.* See "Algæ" (iii) (p. 312).

Vorticella.

- (i) *General Method.* See "Protozoa" (2) (iv) (p. 203), but difficult to avoid contraction.
- (ii) *Narcotize.* Menthol.
- (iii) *Fix.* Suddenly by heat or osmium tetroxide (0.25%).
- (iv) *St.* Methyl green; light green S.F. yellowish; iodine and basic fuchsin; iron hæmatoxylin and orange G.

Whelk. See "Aquatic and Marine Organisms" (p. 128).

Wood. See "Xylem" (below).

Worm. See "Lumbricus" (p. 174).

Xylem.

- (i) *St.* Safranin O (\rightarrow red); counterstain with aniline blue W.S., methyl green, Delafield's hæmatoxylin, basic fuchsin mixed with methylene blue, light green S.F. yellowish in clove oil.

- (ii) *Debenham's method for Xylem of whole plants, or of plant organs.**

Processes must be carried out in covered Petri dishes (at least 4" diameter) which must not contain more than 2 large, or 5 small pieces of tissue. To avoid damage to tissue, liquids must be pipetted in and out of the dishes.

Use either, (a) fresh material,

or (b) material fixed in alcohol (70%),

or (c) dried material. First soak in water at 60° C. (*Not less than 3 weeks*).

1° Clear in lactic acid (70%) or (75%), in oven (58° C.-60° C.). Replace with fresh lactic acid if clearing is prolonged. (*1-3 weeks*).

2° Replace lactic acid with cold glycerine (70%). (*2 days*).

3° Replace with fresh cold glycerine (70%). (*1 day*).

4° Replace with glycerine (50%). (*1 day*).

5° Replace with 2-3 changes of alcohol (50%). (*2-3 days*).

6° If desired to store at this stage,

either, (a) store in alcohol (70%),

or (b) wash in alcohol (70%) and store in

Calberla's fluid. Before proceeding to 7°, wash in alcohol (70%).

(*Several changes. 6-24 hours*).

If not desired to store, proceed to 7°.

* E. M. Debenham, *Annals of Botany*, III, 10, 369. April 1939.
Adapted by kind permission of the Author.

METHODS FOR XYLEM

- 7° Hydrate with alcohol (40%), (30%), (20%), (10%), distilled water. (6 hours each).
- 8° Bleach with strong eau de Javelle, in oven at 40° C. Watch progress of decolorization under microscope. (20 mins.—several hours).
- 9° Wash in cold distilled water.
(Several changes—not less than 12 hours).
If light material tends to float, do not attempt to sink it in the liquid.
- 10° Wash in alcohol (10%).
- 11° If desired to store at this stage,
 - a° Dehydrate to alcohol (60%).
 - b° Store in Calberla's fluid.
(This tends to counteract shrinkage suffered in the bleaching process.)
 - c° After storage, wash in alcohol (70%).
(Several changes. 6–12 hours).
 - d° Hydrate to alcohol (10%).
 If not desired to store, proceed to 12°.
- 12° Stain in fresh ammoniacal fuchsin (special formula, p. 225),
(1–3 days, depending on thickness of material).
Keep dishes in a cool place to avoid loss of ammonia.
- 13° Wash in alcohol (100%).
(2–3 changes, until red colour is washed out of thin-walled tissue and sclerenchyma).
(Xylem → brilliant red, if lignification is sufficiently advanced.)
- 14° Dehydrate in fresh alcohol (100%).
- 15° Mount:
- (a) *Thin specimens.*
- 16° Place a clean slide into the Petri dish of alcohol and float the specimen on to the slide.
- 17° Remove slide and wash with alcohol (100%).
- 18° Draw off alcohol quickly.
- 19° Mount in "Euparal."

20° Cover with a cover-slip. If necessary, remove any bubbles by transferring slide to a hot-plate.

(b) *Thick specimens.*

16° Prepare a cell on a slide by one of the methods suggested under "Mounting" (p. 42).

17° Replace the alcohol in the Petri dish with cedar-wood oil. (*Until specimen is transparent*).

18° Place prepared slide in the dish and float the specimen into the cell.

19° Remove slide from dish and drain oil from cell. (*Until specimen is almost dry*).

20° Fill cell with Canada balsam so that a film spreads on to rim of cell. (Balsam should not be too thin, because solvent may loosen the cell.)

21° Cover. If necessary, remove any bubbles by transferring slide to hot-plate.

Yeast.

(i) *General Method.* See "Protozoa" (2) (iv) (p. 203).

(ii) *St.* (a) *Living.* Nile blue sulphate.

(b) *Fixed.* Delafield's hæmatoxylin.

Zooeæ. See "Aquatic and Marine Organisms" (p. 128).

Zoophytes.

St. Hanstein's rosaniline-violet.

CHAPTER VII

FORMULÆ AND HINTS

- Notes :*
1. For list of abbreviations, see p. 2.
 2. Reagents and stains commonly known by the originator's name are listed under that name. Synonyms are shown in brackets after the name of the stain.
 3. Methods for culture of material will be found in Chapter VIII, p. 291.
 4. Methods for preserving material will be found in Chapter IX, p. 312.

Acetic Acid.

Connective tissue (white fibres disappear, yellow fibres defined); Muscle (nuclei clear, transverse striations indistinct); Nuclei (esp. in fresh or unfixed tissue, e.g. blood, epithelia, liver); Squamous epithelium (temp.).

Glacial acetic acid	1 c.c.
Distilled water	99 c.c.

Acetic-Aniline Blue. See "Hoffmann's Blue" (p. 253).

Acetic-Iodine Green.

Nuclear and plasma.

Iodine green (see p.256)	99 c.c.
Glacial acetic acid	1 c.c.

Acetic-Methylene Blue.*Hydra* (discharges nematocysts).

Methylene blue (see p. 265)	0.2 gr.
Distilled water	99 c.c.
Glacial acetic acid	1 c.c.

Acetic-Methyl Green.

Pollen tubes; Protozoa (kills and stains).

Methyl green (see p. 265). Sufficient to make a clear, weak green-blue solution when added to:

Distilled water.	98.5 c.c.
Glacial acetic acid	1.5 c.c.

Aceto-Carmine.*Nuclear.* (An acid dye.) Stains and fixes. (Differentiate in water.) *Amœba*; Nuclei; *Paramecium*; Protozoa.

Glacial acetic acid	45 c.c.
Distilled water	55 c.c.
Carmine (powdered) (p. 233)	excess.

Dissolve the carmine in the acetic acid, just bring to boil, cool, filter.

Acetone-Xylene.*For rapid clearing from alcohol (90%).*

Acetone (anhydrous)	1 vol.
Xylene	4 vols.

Acetone-Paraffin Wax.

Acetone (anhydrous)	50%
Paraffin wax (m.pt. 50° C.)	50%

Acid Alcohol.

- (1) Alcohol (70%) 100 c.c.
Hydrochloric acid (conc.) 0.5 c.c.
- (2) Specially weak, for Chamberlain's method for fungi:
Wash out a clean vessel with hydrochloric acid (conc.), drain thoroughly, and fill up the bottle with alcohol (70%).

Acid Fuchsin

C.I. No. 692

Plasma. (An acid dye). A good general stain. Blood; Cellulose (counterstain with iodine green); Connective tissue (fresh) (dilute 20 times with Ringer's soln.); Cytoplasm; Chromatophores; may be used as a counterstain for iodine green, methyl green, aniline blue W.S., and malachite green.

May be used in alcoholic or in aqueous solution:

- (a) Acid fuchsin 1 gr.
 Alcohol (50%), (or distilled water) . . . 100 c.c.
- (b) See also van Gieson's stain (p. 248), Orange fuchsin (p. 268), Mallory's triple stain (p. 262), and Pianese IIIb (p. 271).

Acid Green. See "Light Green S.F. Yellowish" (p. 260).

Acid Water.

Distilled water 100 c.c.
 Hydrochloric acid (conc.) 0.5 c.c.

Adhesive for Glass.

- (i) Gum arabic 100 gr.
 Distilled water 250 c.c.
 add:
 Aluminium sulphate (cryst.) 2 gr.
 Distilled water 20 c.c.
- (ii) See also "Gum for Labels" (p. 252).

Albumen. See "Egg Albumen" (p. 243).

"Albumen-Glycerine." See "Egg Albumen" (p. 243).

Alcohol (Ethyl).

- (i) *Absolute (100%) will not remain absolute if the bottle is left without a stopper or if anyone breathes or sneezes into the bottle.*
- (ii) Absolute may be prepared from ordinary methylated (industrial) spirits (which is about 95% pure) by standing the meths. on anhydrous copper

sulphate (two or three changes) *for several days*, filtering, distilling over caustic potash, refluxing on calcium metal *until the contents of the flask appear white*, fractionating over freshly prepared quick lime and collecting at 78° C. only.

A calcium chloride tube should be inserted at the top of the reflux condenser, and at the open end of the receiver in the final distillation.

Test a small quantity for the presence of water by adding either, a piece of calcium carbide (a smell of acetylene indicates that water is present), or, a small quantity of turpentine (any water will be visible under the microscope).

- (iii) Grades of alcohol lower than 95% may be prepared from ordinary *industrial* methylated spirit after first standing it on anhydrous copper sulphate (two or three changes) for several days and filtering. On the assumption that this dehydrated industrial spirit will be 95%, the Table for the Dilution of Liquids (p. 240) may be used for finding the volumes of water to add to make up various grades.
- (iv) It is a useful saving of time to have Winchester-quart bottles, graduated to hold 2,000 c.c. each, of different strengths of alcohol. This leaves room for thorough shaking. See that the bottles are clean, pour in the required proportion of alcohol (95%), fix a label on the bottle, and mark the label with a horizontal line at the level the alcohol reaches, adding the words "*Alcohol (95%) to this line →.*" Now, without yet shaking, add the required proportion of distilled water, fix another label, mark the label with a horizontal line at the level the mixture reaches and the words "*Add distilled water to this line →.*" Finally label the bottle with the strength of alcohol it contains. Thorough shaking is now essential.
- (v) *For dissolving intercellular substances prior to teasing.*
30%.

(vi) *For fixation.*

(a) Rapid fixation of animal tissue 100%

(b) Slow fixation of animal tissue.

Alcohol (90%) . . . 1 part.

Water . . . 2 parts.

Do not allow the tissue to remain in the fixative for longer than 24 hours.

(c) Fixation of plant tissue. 90%

After fixation, wash in alcohol.

(vii) *For hardening* . . . 90%(viii) *For preserving.*

(a) general tissue. . . 70%

(b) sponges . . . 75%

(c) worms . . . 90%

Alcohol-Formalin. See "Formalin-Alcohol" (p. 247).

Alcohol-Soluble Eosin. See "Ethyl Eosin" (p. 246).

Alcohol-Xylene.

Alcohol (100%) . . . 50 c.c.

Xylene . . . 50 c.c.

Alizarin Red S. C.I. No. 1034.

Bone (Dawson's method).

Alizarin red S. . . 0.1 gr.

Potassium hydroxide (1%) . . 1,000 c.c.

Alkaline Alcohol.

Alcohol (90%) . . . 99.5 c.c.

Ammonium hydroxide (Sp. Gr. 0.880). 0.5 c.c.

Alkaline Water. See "Tap-Water Substitute" (p. 285).

Alkannin. C.I. No. 1240.

Cork (→ red); Cuticle (→ red); Fats (→ deep red);
Protoplasm (→ pale rose red) ($\frac{1}{2}$ hour); Resin (→ deep red) (1 hour).Satd. solution of the roots of *Alcanna tinctoria* in alcohol (50%).

Alum Carmine. See "Mayer's Alum Carmine" (p. 264).

Amidol.

To reduce silver nitrate (p. 152). 1% aq. soln.

Ammoniacal Fuchsin.

Bundle ends; Lignified tissue (\rightarrow red).

- (i) Basic fuchsin (see p. 228) . . . 5.0% in
Ammonium hydroxide . . . 0.88 sp. gr.

Add the basic fuchsin to the ammonia until a permanent straw colour is obtained. Filter.

- (ii) *Special formula for Debenham's method for xylem*
(p. 217).

Basic fuchsin (Gurr's Special). Filtered sat.
soln. in alcohol (100%).

Ammonium hydroxide . . . 0.88 sp. gr.

Add the ammonium hydroxide from a dropping funnel, in a steady stream, into the basic fuchsin solution until the liquid is a pale yellow. Shake continuously while mixing.

Use within 24 hours.

Ammonium Dichromate.

For fixing and hardening nervous tissue.

Ammonium dichromate . . . 10 gr.

Distilled water . . . 1,000 c.c.

Ammonia-Hæmatin.

Cladophora sp. (after picric acid fixation).

- (i) 1° Place a few crystals of hæmatoxylin (see p. 252)
in a few c.c.s of distilled water.
2° Pass ammonia vapour through the liquid until the
hæmatoxylin dissolves to give a violet solution.
3° Dilute the solution considerably with distilled
water.

(ii) *Use* :

- 1° Stain the tissue in this solution for several hours (until the tissue is slightly overstained).
- 2° Then wash in distilled water until the preparation is of the desired colour.

Anderson's Iron Alum Hæmatoxylin.

Nuclear. General animal histology ; *Paramecium* (nuclear detail) ; *Vorticella* (with orange G).

N.B. Hæmoglobin has a great affinity for iron hæmatoxylin: do not mistake red blood corpuscles for nuclei.

Solution 1.

Hæmatoxylin (see p. 252)	0.5 gr.
Alcohol (50%)	100 c.c.
Calcium hypochlorite (2% aq.)	3 c.c.

Solution 2. (The mordant.)

Iron alum	4 gr.
Distilled water	100 c.c.
Sulphuric acid (conc.)	3 c.c.

Just before use, mix two parts of Solution 1 with one part of Solution 2. Mixture keeps for a few hours only.

Iron alum can be used to differentiate.

Aniline Blue W.S. (Cotton Blue; Water Blue.) C.I. No. 707.

Plasma. (An acid dye.) Dissolves from cell walls in glycerine. Counterstain to safranin O. Algæ (with Magdala red or phloxine B); Callose of sieve plates; Cellulose; Cell contents (→ blue); Fungi (with Magdala red); *Spirogyra*; Unlignified tissue. Double stain with acid fuchsin.

May be used in alcoholic or in aqueous solution.

(a) Aniline blue W.S.	1 gr.
Distilled water [or alcohol (70%)].	99 c.c.

(b) *For algae—Chamberlain's method.*

Aniline blue W.S.	1 gr.
Alcohol (90%)	99 c.c.

(c) *For algae and fungi.*

(i) Aniline blue W.S.	0.4 gr.
Distilled water	100 c.c.
(ii) Aniline blue W.S.	0.4 gr.
Lacto-phenol	96.6 c.c.

(d) *For fungal hyphae in host tissue (Godwin's method).*

[See "Fungi" (iv) (c) (2) (p. 163).]

Aniline blue W.S.	0.25 gr.
Lacto-phenol (special formula for	

Godwin's method—see p. 259) 99.75 gr.

(e) See also Hoffmann's blue (p. 253), Mallory's triple stain (p. 262), and picric-aniline blue W.S. (p. 272).

Aniline Crystal Violet (Aniline Gentian Violet).

Bacteria; Plant tissue; Mitosis.

Solution A.

Crystal violet (see p. 238)	2.5 gr.
Ethyl alcohol (95%)	12.0 c.c.

Solution B.

Aniline	2 c.c.
Distilled water	98 c.c.

Shake and allow to stand for a few minutes; then filter.

Mix solutions A and B.

Aniline-Gentian Violet. See "Aniline Crystal Violet" (above).**Aniline Red.** See "Basic Fuchsin" (p. 228).**Aniline-Water-Fuchsin.** See "Ehrlich's Aniline-Water-Fuchsin" (p. 243).

Aniline Sulphate.

Lignin (temp.) (\rightarrow yellow). This is a specific stain for lignin.

Aniline sulphate	1 gr.
Alcohol (70%)	89 c.c.
Sulphuric acid	$\frac{N}{10}$.	.	.	10 c.c.

Arsenical Soap. See "Bécoeur's Arsenical Soap" (p. 229).

Babes's Safranin.

Safranin O (see p. 280)	.	.	.	excess
Aniline oil	.	.	.	2 c.c.
Alcohol (50%)	.	.	.	100 c.c.

Warm the mixture to between 60°–80° C. Filter through a wet filter. Will keep 1–2 months.

Basic Fuchsin. (Aniline red; Magenta; Rosaniline.) C.I. No. 677.

Nuclear. (A basic dye.) Bacteria; *Chara* (temp. with iodine); Hyphæ protoplasm (with iodine); Lignin (\rightarrow red); *Nitella* (with iodine); *Protococcus* (with iodine); *Vorticella*; Yeast (mix the stain with methylene blue).

(a) Basic fuchsin	.	.	.	0.1 gr.
Distilled water	.	.	.	160 c.c.
Alcohol (70%)	.	.	.	1 c.c.

(b) See also, ammoniacal fuchsin (p. 225), Ehrlich's aniline-water-fuchsin (p. 243), Hanstein's rosaniline violet (p. 252), Weigert's stain (p. 287), Ziehl's carbolic-fuchsin (p. 290).

Bause's Ringing Varnish. See "Ringing Media" (i) (p. 278).

Bécoeur's Arsenical Soap (poison).*For preservation of skins.*

White soap 2 lb.

Cut up and boil. Add

Potassium carbonate (anhydrous) . . . 12 oz.

Calcium carbonate ("whiting") . . . 4 oz.

Mix thoroughly. When nearly cold add

Arsenious oxide 2 lb.

Camphor 5 oz.

(The camphor should be triturated with
alcohol before addition.)

Pour into jars for subsequent use.

Benzene-Phenol. See "Xylene-Phenol" (p. 289) and
substitute benzene for xylene.**Benzol.** For clearing, use benzene-phenol (above).**Berlese's Fluid.**

Chloral hydrate 160 gr.

Gum arabic 15 gr.

Glucose syrup 10 c.c.

Acetic acid (dil.) 5 c.c.

Distilled water 20 c.c.

Best's Carmine.

For glycogen (in wax-embedded sections).

Carmine 2 gr.

Potassium carbonate 1 gr.

Potassium chloride 5 gr.

Distilled water 60 c.c.

Warm gently (5 mins.). Cool. Add

Ammonium hydroxide 20 c.c.

Best's Differentiator.

For use with Best's carmine.

Ethyl alcohol (100%) 80 c.c.

Methyl alcohol (100%) 40 c.c.

Distilled water 100 c.c.

Bismarck Brown Y. (Vesuvian.) C.I. No. 331.

Nuclear. (A basic dye.) Bacteria; Cellulose; Living organisms; Plant tissue.

May be used in solution in isotonic saline (for living organisms), in water, or in alcohol.

Bismarck brown Y	0.3 gr.
Isotonic saline [or water, or alcohol (95%)]	100 c.c.

Borax Carmine (Grenacher's Alcoholic).

Nuclear. (A neutral dye.) A good general stain for sectioned and bulk animal tissue, and bulk plant tissue; Animal tissue; Small entire objects; Chick embryo; *Fasciola*; *Fucus*; *Glochidium*; *Helix* (nerve collar); *Lumbricus* (ovary); Muscle; *Nereis* (parapodia) (with picric acid); Nuclei (\rightarrow pink); *Obelia*; *Opalina*; *Paramecium*; *Periplaneta* (fat body; leg muscle; salivary duct) (followed by picric acid alc.); Plant tissue in bulk; Spinal cord.

(a) Borax	4 gr.
Carmine (see p. 233)	3 gr.
Distilled water	100 c.c.
Heat the above, then add	
Alcohol (70%)	100 c.c.

Stand for *two days*. Filter.

(b) See also Aceto-carmine (p. 221); Mayer's alum-carmine (p. 264).

Borrel's Methylene Blue.

Nerve tissue; Mucin.

Silver nitrate (1%). Ppt. by dil. sodium hydroxide soln. Filter and wash ppt. thoroughly. Add silver ppt. to

Methylene blue (1% aq.). Boil 10 mins. until colour goes dark *violet*. Cool. Filter. Dilute with 4 times its vol. of distilled water.

Bouin's Fluid (Picro-formo-acetic).

A good fixative for use before Heidenhain's iron hæmatoxylin.

Picric acid (aq. satd.)	. . .	75 c.c.
Glacial acetic acid	. . .	5 c.c.
Formaldehyde (40%)	. . .	25 c.c.

Breinl's Stain.

For cytological work on fungi. A method involving staining with iodine (p. 256), and counterstaining with safranin O (p. 280), polychrome methylene blue (p. 273), and orange tannin. See "Fungi" (iv) (b) (4) (p. 161) for method; and the pages mentioned for special formulæ of stains.

Bristol's Solution.

For culture of algae.

Potassium dihydrogen phosphate	. . .	1 gr.
Sodium nitrate	. . .	1 gr.
Magnesium sulphate	. . .	0.3 gr.
Calcium chloride	. . .	0.1 gr.
Sodium chloride	. . .	0.1 gr.
Ferric chloride	. . .	trace.
Distilled water	. . .	1,000 c.c.

Brown's Medium.

For culture of fungi.

Agar-agar	. . .	2.00 gr.
Asparagen	. . .	0.20 gr.
Glucose	. . .	0.20 gr.
Magnesium sulphate	. . .	0.075 gr.
Potassium phosphate	. . .	0.125 gr.
Starch	. . .	1.00 gr.
Distilled water	. . .	100 c.c.

Browne's Soap.*For preservation of skins.*

White curd soap	1 lb.
Calcium carbonate ("whiting")	3 lb.
Boil together. Whilst still hot, add	
Bleaching powder	1½ oz.
(Avoid inhaling the fumes while the mixture is hot.)	
When cold, add	
Tincture of musk	1 oz.

Cajal's Fixative (de Fano's modification).*For fixation in Cajal's method for Golgi bodies.*

Cobalt nitrate	1 gr.
Formaldehyde (40%)	15 c.c.
Distilled water	100 c.c.

Calberla's Fluid.*For storage of fixed and partially dehydrated fungi and other plant tissue.*

Alcohol (100%)	30 c.c.
Glycerine (pure)	30 c.c.
Distilled water	30 c.c.

Canada Balsam in Benzene.

As for Canada balsam in xylene (below)—but using benzene as solvent.

Canada Balsam in Xylene.Heat the resin supplied *until, on cooling, it becomes brittle.*

Dissolve in xylene till a thin solution is obtained.

Should, preferably, be prevented from becoming acid.

This is difficult—but the following may be tried:

(i) Stir with calcium carbonate powder or anhydrous sodium carbonate and filter. Place filtrate near a source of heat (*care*) to evaporate to right consistency.

(ii) Keep a piece of calcium carbonate or a small marble in the bottle.

(iii) Paint containers black outside, to exclude light.

Carazzi's Hæmatoxylin.*

May be counterstained with eosin Y (0.5% aq.).

Hæmatoxylin	0.75 gr.
Potassium alum	37.50 gr.
Potassium iodate	0.15 gr.
Glycerine	250 c.c.
Distilled water	600 c.c.

Do not heat to dissolve.
There is no need to allow to ripen.

Carbol-Fuchsin. See "Ziehl's carbolic-fuchsin" (p. 290).

Carbol-Methylene-Blue.**Bacteria.**

Methylene blue (see p. 265)	1.5 gr.
Phenol	5.0 gr.
Alcohol (100%)	10 c.c.
Distilled water	100 c.c.

Carbol-Thionin.

Fungal mycelium in host plant. (Mycelium → violet purple; lignified tissue → blue.)

Thionin (see p. 286)	1 gr.
Phenol	5 gr.
Distilled water	100 c.c.

Filter; dilute before use 50/50 with distilled water.

Carbol-Turpentine.†

Phenol (melted)	2 vols.
Turpentine (rectified)	3 vols.

Carmalum. See "Mayer's Carmalum" (p. 264).

Carmine. C.I. No. 1239. See Aceto-carmine (p. 221), borax carmine (p. 230), iron-aceto-carmine (p. 256), Mayer's alum carmine (p. 264), and picro-carmine (p. 272).

* Langeron, *Précis de Microscopie*. 1925.

† After E. J. Durand, *Phytopathologist*, I, 129, quoted by Strasburger, *Handbook of Practical Botany* (English edn.), Allen and Unwin.

Carnoy's Fluid.

- (i) Ethyl alcohol (100%) 75 c.c.
 Acetic acid (glacial) 25 c.c.
 (ii) Do not use for fixing plant material.

Carragheen Solution. (Irish Moss).*

Dry and bleach some seaweed (*Chondrus* and/or *Gigartina*). Place a teaspoonful in half a test-tube of boiling water. Boil gently for a time. When cooled the viscous fluid becomes a yellow jelly.

Carrot.

For holding material during section cutting. Cut a supply into suitable sizes and store in alcohol (70%).

Cedar-Wood Oil.

When ordering, specify whether for clearing tissue or for oil-immersion objectives. The former is cheaper.

Cellophane—gum for.

Add 15% of glycerine to any good glue, gelatine or gum, or to waterglass.

Cellulose Varnish.

Use "Brushing Belco."

Cement. See also "Ringing Media" (p. 278).

- (i) *Apáthy's cement for glycerine mounts.*†
 Canada balsam 1 part.
 Paraffin wax (m.pt. 60° C.). 1 "

Heat in a porcelain vessel until the mixture turns golden and no longer gives off turpentine vapours.

To use—give one application of the warmed cement on a glass rod or spatula.

- (ii) Shellac dissolved in alcohol. (Is immersion-oil proof.)

* After Stork and Renouf, *Fundamentals of Biology*, Murray.
 † After Lee, *Microtomist's Vade-Mecum*, Churchill.

(iii) "Robbialoid" black enamel (three coats). (Is immersion-oil proof.)

(iv) *Tolu Balsam Cement*.*

Tolu balsam 2 parts.

Canada balsam. 1 part.

Shellac (satd. soln. in chloroform) 2 parts.

Chloroform—to bring to a syrupy consistency.

(v) *Waterproof Cement for Sealing Specimen Jars*.

Use pitch. (This may be collected from the tops of old accumulators and dry batteries.) Melt the pitch, and apply and smooth off with an old scalpel, heated. Cover joints with "Passe-par-tout."

(vi) "Coron." † If necessary, thin with benzene.

Chloral Hydrate.

(i) Chloral hydrate 64 gr.

Water 40 c.c.

(ii) 0.1% aq. for narcotization of small marine animals.

Chlorazol Black E. C.I. No. 581.

Bulk and section staining. For use and results see

"Animal Tissue" (v) (I) (d) (p. 119).

‡Chlorazol black E (Biological quality) Satis.

Alcohol (70%) 100 c.c.

Differentiate with dilute "Milton" (p. 266).

Chlor-Zinc-Iodine. See "Schulze's Solution" (p. 282).

Chrom-Acetic.

(i) *General Formula* for fixation of plant tissue.

Chromic "acid" 1 gr.

Acetic acid (glacial) 1 c.c.

Distilled water 98 c.c.

* After Lee, *Microtometist's Vade-Mecum*, Churchill.

† Obtain from T. Gerrard & Co.

‡ Obtain from Sales Office, Imperial Chemical Industries, Ltd., London.

(ii) *Formula for algae.*

Chromic "acid"	0.7 gr.
Acetic acid (glacial)	0.3 c.c.
Distilled water	99 c.c.

Fix for a few minutes.

(iii) *For filamentous algae, fungi and prothalli.* (Another formula.)

Chromic "acid"	2.5 parts.
Acetic acid (glacial)	5.0 "
Water.	72.5 "

(iv) *For root tips, ovaries of plants.*

Chromic "acid" (10%)	7 parts.
Acetic acid (10%)	10 "
Water.	83 "

(v) *For woody tissue, leaves.*

Chromic "acid" (10%)	10 parts.
Acetic acid (10%)	10 "
Water	80 "

Fix several hours.

(vi) *Marine formula, for seaweeds.*

Chromic "acid"	1 gr.
Acetic acid (glacial)	0.4 c.c.
Sea-water	400 c.c.

Chromic Acid.

- (i) *For hardening eyes.* 0.25% aq.
- (ii) *For fixing animal tissue for cytological purposes.* 0.5%.
- (iii) *For teasing small animals and for fixing plant tissue for histological purposes.* 1% aq.
- (iv) *For removing grease from glassware* (see p. 249). Add excess potassium dichromate to conc. sulphuric acid.

Chrome-Osmium.

Chromic "acid" (0.25%)	9 parts.
Osmium tetroxide (1%).	1 part.

Chrome-Osmium-Acetic. See "Flemming's fluid" (p. 247).

Cleaning Slides. See "Glassware" (p. 249).

Clip for Holding Slides. See "Slide-holder" (p. 283).

Cocaine Hydrochloride.

For narcotizing Rotifers.

- | | | |
|------------------|---|---------|
| (a) 1% aq. soln. | | |
| (b) 2% aq. soln. | . | 30 c.c. |
| Alcohol (96%) | . | 10 c.c. |
| Distilled water | . | 60 c.c. |

Congo Red. C.I. No. 370.

For rusts, especially after acetic-alcohol fixation and staining with hæmatoxylin. Colours walls of parasite but not host.

- | | |
|------------------------------|--------------|
| Congo red | 1 gr. |
| Distilled water | 100 c.c. |
| Ammonium hydroxide | A few drops. |

Corallin Red. C.I. No. 726.

A temp. stain. Callose (→ deep red); Cambium (→ pale yellow); Cellulose; Lignin (→ deep red); Phloem (→ pale reddish-yellow); Sieve plates (→ deep red); Starch (→ rose).

Saturated solution in sodium carbonate (4% aq.). Add a little camphor.

"Corrosive-Acetic" (Poison).

(a) *For general animal histology.*

- | | |
|---|----------------|
| Mercuric chloride (aq. satd.) | 95 c.c. |
| Acetic acid (glacial) | 5 c.c. |
| For fixing small animals. | (3-5 mins.). |
| For fixing larger pieces of tissue. | (10-15 mins.). |

(b) *For smears of seminal vesicles of Lumbricus.*

- | | |
|---|----------|
| Mercuric chloride (aq. satd.) | 100 c.c. |
| Acetic acid (glacial) | 1 c.c. |

Wash in alcohol (70%) to which a few drops of iodine solution (p. 256) have been added. If colour disappears add more iodine. (2 mins. for small tissues).

(c) *For desmids.*

Mercuric chloride	3 gr.
Acetic acid (glacial)	3 c.c.
Alcohol (50%)	100 c.c.
Use hot solution.	

“Corrosive-Formaldehyde” (Poison).

Mercuric chloride (aq. satd.)	90 c.c.
Formaldehyde (40%)	10 c.c.

“Corrosive Sublimate.” See “Mercuric Chloride” (p. 264).

Cotton Blue. See “Aniline Blue W.S.” (p. 226).

Cotton Red. See “Safranin O” (p. 280).

Cover-slips—To Clean. See “Glassware—To Clean” (p. 249).

Crystal Violet (Gentian Violet). C.I. No. 681.

Gives a deep blue violet (cf. Methyl violet 2B, p. 265).

Nuclear. (A basic dye.) Bacteria; Epithelia; Fungus (nuclei); Mitotic figures; Nuclei (plant) (counterstain with Gram's iodine and orange G in clove oil); Spermatozoids.

Can be differentiated with clove oil.

(a) *For general use, and for Newton's method for mitotic figures* (iii) (b) (p. 179).

Crystal violet	1 gr.
Distilled water	99 c.c.

(b) *For mitotic figures, method* (iii) (c) (p. 179).
Saturated solution of crystal violet in distilled water.

(c) *For other cytological methods.*

Crystal violet	1 gr.
Alcohol (20%)	100 c.c.

(d) A 1% solution in *clove oil* is often useful.

(e) See also “Aniline Crystal Violet” (p. 227).

Culture Media.

Only named media are listed in this Chapter. Un-named media are listed (under the name of the organism for which they are to be used) in Chapter VIII (p. 291), to which reference should be made.

Cyanide. See "Killing Bottle" (p. 258).

Cyanin (Quinoline blue). C.I. No. 806.

Plasma. Lignin (→ blue) (counterstain with Bismarck brown Y). Also a counterstain for erythrosin bluish, e.g. lichens (algæ → blue; fungus filaments → red).

Aniline oil	3 c.c.
Distilled water	97 c.c.
Shake well, add		
Alcohol (100%)	25 c.c.
To every 100 c.c. of above solution add		
Cyanin	1 gr.

Dahlia. See "Hofmann's W.S. violet" (p. 253).

Dammar. See "Gum Dammar" (p. 251).

Decalcifying Fluid.

(i) *For General Work.*

Formaldehyde (5% aq.)	100 c.c.
Nitric acid (1.4 sp. gr.)	7.5 to 15 c.c.
Wash in sodium sulphate (aq.)	(p. 283).	

(ii) *For Starfish.*

Alcohol (70%)	99 c.c.
Hydrochloric acid (conc.)	1 c.c.

(iii) *For Shelled Protozoa.*

First fix, and then decalcify in acid-alcohol, viz.:		
Nitric acid (conc.)	1 c.c.
Alcohol (70%)	99 c.c.

Delafield's Hæmatoxylin.

Nuclear. A good general stain. (A basic dye.) Cytoplasm (selective); Cellulose (counterstain to safranin O) (→ purple); Cell walls (unlignified) (counterstain with eosin Y, or erythrosin bluish); Chromatin (→ purple); Epithelia; *Fasciola*; *Hydra*; *Lumbricus* (entire); Mitotic figures; Mosses; Mycelia in wood; Nuclei (animal and plant) (→ blue); Plant tissue [fresh sections; fixed sections (with safranin O); thick objects; whole mounts; general histology]; Ovary; Pituitary; Protozoa; Sieve Plates; Spinal Cord; Suprarenal body; Testis; Thyroid; *Trypanosoma*; Yeast. May be followed by picric acid where chitin or horn is to be stained.

Ammonia alum (aq. satd.)	400 c.c.
Hæmatoxylin [16% in alcohol (100%)]	25 c.c.

Stand 4 *days* (in a flask plugged with cotton wool) exposed to light. Filter. Add:

Glycerine	100 c.c.
Methyl alcohol	100 c.c.

Place in a warm, light situation for 6 *weeks*. Ripening more rapid by adding a small quantity of hydrogen peroxide. Improves with age. As it ripens, dilute with ammonia alum (aq. satd.). If the hæmatoxylin is *very* old it will not "blue" with tap-water. If in this red condition, add a few drops of ammonium hydroxide to the stock.

Dilution of Liquids, Table for.

Note: The figures in this table take no account of any possible change in volume due to the mixture of the two liquids.

		Percentage Strength of Original Liquid.																		
		100	96	95	90	85	80	75	70	60	50	40	30	20	15	10	8	5	4	3
Percentage Strength of Liquid Required and Volumes of Original Liquid to be taken.	95	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	90	10	6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85	15	11	10	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80	20	16	15	10	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	75	25	21	20	15	10	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	70	30	26	25	20	15	10	5	-	-	-	-	-	-	-	-	-	-	-	-
	60	40	36	35	30	25	20	15	10	-	-	-	-	-	-	-	-	-	-	-
	50	50	46	45	40	35	30	25	20	10	-	-	-	-	-	-	-	-	-	-
	40	60	56	55	50	45	40	35	30	20	10	-	-	-	-	-	-	-	-	-
	30	70	66	65	60	55	50	45	40	30	20	10	-	-	-	-	-	-	-	-
	20	80	76	75	70	65	60	55	50	40	30	20	10	-	-	-	-	-	-	-
	15	85	81	80	75	70	65	60	55	45	35	25	15	5	-	-	-	-	-	-
	10*	90	86	85	80	75	70	65	60	50	40	30	20	10	5	-	-	-	-	-
	8	92	88	87	82	77	72	67	62	52	42	32	22	20	7	2	-	-	-	-
	5†	95	91	90	85	80	75	70	65	55	45	35	25	15	10	5	3	-	-	-
	4	96	92	91	86	81	76	71	66	56	46	36	26	16	11	6	4	1	-	-
	3	97	93	92	87	82	77	72	67	57	47	37	27	17	12	7	5	2	1	-
	1	99	95	94	89	84	79	74	69	59	49	39	29	19	14	9	7	4	3	2
		Volumes of Diluent to be added.																		

Notes.—1. Heavy type indicates strengths of alcohols in most common use. Industrial methylated spirit reckoned as alcohol (95%).

2. Italics indicate strengths of formaldehyde most common. [Formalin = formaldehyde (40%).]

* To make "Strong formol-saline" use sodium chloride soln. (0.9% aq.) as the diluent for formaldehyde (10%).

† To make "Weak formol-saline" use sodium chloride soln. (0.9% aq.) as the diluent for formaldehyde (5%).

[Notes continued overleaf.

3. Intermediate figures in the table may be calculated as follows:

From the percentage strength of the original liquid subtract the percentage strength of the liquid required. The difference is the number of volumes of diluent which must be added to that number of volumes of original liquid which is indicated by the same number as denotes the percentage strength of the liquid required.

e.g. Strength of original liquid	84.7%
Strength of liquid required	33.5%
Volumes of diluent to be added	51.2 vols.
Volumes of original liquid to be taken.	33.5 vols.

Dox's Agar.

Magnesium sulphate	0.5 gr.
Potassium acid phosphate	1.0 gr.
Potassium chloride	0.5 gr.
Ferrous sulphate	0.01 gr.
Sodium nitrate	2.0 gr.
Cane sugar	15.0 gr.
Agar-agar	20.0 gr.
Water	1,000 c.c.

Boil all together, filter, tube, sterilize as for "Potato Agar" (p. 274).

Dreft. See "Glassware—To Clean" (ii) (b) (p. 249).

Dung Agar.

For culture of fungi.

Dung (cow, horse, or rabbit) 1,000 gr.

Soak the dung in cold water (3 days). Decant and dilute till straw-coloured. For every 100 c.c. of diluted extract, add:

Agar-agar 2.5 gr.

Eau de Javelle.

- (i) Bleaching powder 20 gr.
 Water 100 c.c.

Stand for some hours and add :

- Potassium carbonate 15 gr.
 Water 100 c.c.

Filter. If a film forms on the surface on exposure to air, add more potassium carbonate solution and filter.

- (ii) *Formula for use with Debenham's method for xylem*
 (p. 217).

- Bleaching powder satis.
 Water 100 c.c.

Allow the bleaching powder to dissolve for at least 24 hours. Filter off undissolved solid.

To the solution add the following, freshly made : Potassium oxalate (conc. soln.) in distilled water, until no further precipitate is formed. Filter off the eau de Javelle as required.

Egg Albumen.

- White of egg 50 c.c.
 Glycerine 50 c.c.
 Salicylate of soda 1 gr.

First shake the white of egg with a few drops of acetic acid (dil.). Add the other ingredients and shake well together. Filter (*long time*) (if necessary on a filter pump) into a clean bottle.

Ehrlich's Aniline-Water-Fuchsin.

- Bacteria spores.
 Distilled water 100 c.c.
 Aniline oil 5 c.c.

Shake up, and stand for 5 mins. Filter through a paper wetted with distilled water. Filtrate must be water clear.

[Continued overleaf.

Add:

Sodium hydroxide (1%) 1 c.c.
then,

Basic fuchsin (see p. 228) 5 gr.

Shake. Stand for 24 hours. Use within 2 days.

Note: Methyl violet 2B, crystal violet, or methylene blue may be used in place of basic fuchsin.

Ehrlich's Hæmatoxylin.

Nuclear. For general animal histology. (A basic dye.)
Kidney, Liver, Testis [all counterstained with eosin Y (alc.)]; *Monocystis*.

Hæmatoxylin 2 gr.

Alcohol (100%) 100 c.c.

add:

Distilled water 100 c.c.

Glycerine 100 c.c.

Acetic acid (glacial) 10 c.c.

Potash alum excess.

Leave the whole in a large bottle in sunlight. Remove stopper for a few minutes. Replace. Shake. Leave open. Repeat this over several weeks.

Elder Pith.

For holding small pieces of tissue during sectioning. Keep a small stock in alcohol (70%). Cuts more easily than when wetted just before use.

Eosin-Hæmatoxylin. See "Renaut's Eosin-Hæmatoxylin" (p. 278).

Eosin W.S. See "Eosin Y" (below).

Eosin Y (i.e. yellowish) (Eosin W.S.; Water soluble eosin).
C.I. No. 768.

Plasma. (An acid dye.) For general animal and plant histology. Aleurone grains—with crystalloids (use alc. soln.); bacteria-containing tissue; blood; cell

walls—unlignified (counterstain to Delafield's hæmatoxylin); connective tissue; cytoplasm (\rightarrow red); kidney (counterstain to Ehrlich's hæmatoxylin); nuclei (plant and animal); protoplasm (especially plant); sieve tubes; testis (counterstain to Ehrlich's hæmatoxylin). May be used as a counterstain to Delafield's hæmatoxylin (see p. 120 for some results), iodine green, and Loeffler's methylene blue.

This dye [to be distinguished from ethyl eosin (= alcohol soluble eosin)] is soluble in either water or alcohol.

(a) *Alcoholic solution* :

Eosin Y	1 gr.
Alcohol (75%)	99 c.c.

(b) *Aqueous solution* :

Eosin Y	1 gr.
(As counterstain to Carazzi's hæmatoxylin, use only 0.5 gr. eosin Y.)	
Distilled water	99 c.c.

(c) *As counterstain after a basic dye.*

Eosin Y	0.5 gr.
Alcohol (95%)	25 c.c.
Distilled water	75 c.c.

(d) *In clove oil.*

Clove oil with eosin Y to saturation.

(e) *For use after treating with potassium ferrocyanide for iron.*

Eosin Y	1.0 gr.
Alcohol (30%)	100 c.c.

(f) See also, Mann's stain (p. 263), Renaut's eosin-hæmatoxylin (p. 278), and xylene-eosin Y (p. 288).

N.B. Eosin is rapidly washed out by 70% and 90% alcohol : overstain sections slightly and, if alcohol is used for dehydration, dehydrate for 1 *min.* in alcohol (96%), and 1 *min.* in alcohol (100%).

Eosin Yellowish. See "Eosin Y" (p. 244).

Erythrosin Bluish. C.I. No. 773.

Cell walls (counterstain to Delafield's hæmatoxylin);
Cytoplasm; Protoplasm (esp. plant). May be counter-
stained with cyanin, or iodine green.

(a) *Alcoholic soln.*

Erythrosin bluish	1 gr.
Alcohol (90%)	99 c.c.

(b) *Aqueous soln.*

Erythrosin bluish	1 gr.
Distilled water	99 c.c.
Formaldehyde (40%)	4-5 drops.

(c) See also Xylene-eosin Y (p. 288).**Ethyl Eosin. (Alcohol soluble eosin.)**

This stain has no special advantages for the beginner.

Farrants' Medium.

White gum arabic	30 gr.
Distilled water	30 c.c.

Do not heat. Stir occasionally.

Add:

Arsenious oxide	0.01 gr.
Glycerine	30 c.c.

Strain through clean flannel if necessary. Keep in
stoppered bottle with piece of camphor.

Material should be soaked for *a few minutes only*.

Fat ponceau G. See "Sudan III" (p. 284).

Fat ponceau R. See "Sudan IV" (p. 284).

Filter Paper.

(i) Use technical quality and buy in sheets 62 cms. \times 62 cms.

(ii) Cut into pieces 3" \times 2" for irrigation, etc.

Fixatives.

Refer to table under technique of fixation (pp. 16-21).
Only named fluids are listed in this chapter.

Flemming's Fluid. (Chrome-osmium-acetic.)

Chromic "acid"	0.5 gr.
Osmium tetroxide	0.2 gr.
Acetic acid (glacial)	0.2 gr.
Distilled water	99 c.c.

Use at half strength for fixing fungi and rotifers.

Formaldehyde, Dilution of. See "Dilution of Liquids, Table for" (p. 240).

Note: Keep a lump of calcium carbonate at the bottom of the formaldehyde bottle to help to neutralize the formic acid produced on oxidation; or neutralize with sodium carbonate before use.

Formalin-Alcohol.

Alcohol (70%)	100 c.c.
Formaldehyde (40%)	6 c.c.

Fixation should be for 15 *mins.* for small and delicate pieces of tissue, and 12 *hours* for large pieces of coarser tissue. Wash in alcohol (70%).

Formalin-Dichromate.

For hardening brains:

Potassium dichromate (1% aq.)	50 c.c.
Formaldehyde (8%)	50 c.c.

Leave the brain in the mixture for 2-3 *weeks*.

Wash in running water for 1 *day*. Preserve in formalin (5%).

Formo-Acetic Alcohol.

Especially useful for fixation and preservation of algæ.

Formaldehyde (40%)	5 c.c.
Acetic acid (glacial)	7 c.c.
Alcohol (70%)	90 c.c.

Formol-Saline.

See footnotes (*) and (†) below "Table for Dilution of Liquids" (p. 241), and use sodium chloride (0.9% aq.) as the diluent for formaldehyde.

Gatenby's Fluid.*

For removal of albumen from eggs of *Rana*.

Potassium dichromate (2%)	100 c.c.
Chromic acid (1%)	100 c.c.
Nitric acid (conc.)	6 c.c.

Gentian Violet. See "Crystal Violet" (p. 238).

Giemsa's Stain.†

Blood and blood parasites. See "Blood" (iv) (c) (p. 135) for use and results.

(i) Azure A	1.9 gr.
Methylene blue	0.4 gr.
Methylene blue eosinate	1.5 gr.
Glycerine (pure)	125.0 gr.
Methyl alcohol (pure and acetone-free)	375.0 gr.

Store in a clean glass-stoppered bottle.

(ii) If not prepared in the laboratory, purchase Gurr's or Grüber's.

van Gieson's Stain.

Plasma. Cartilage; Blood Vessels; Connective tissue (→ red); Elastic fibres (→ yellow); Epithelia (→ yellow); Muscle (→ yellow); see also results when used as a counterstain to iron hæmatoxylin.

Acid fuchsin (1% aq.) (see p. 222) . . . 5 c.c.

Boil well. Filter. Add

Picric acid (aq. satd.) (see p. 271) . . . 100 c.c.

* Bolles-Lee, *Microtometist's Vade-Mecum*, Churchill.

† After Hartridge and Haynes, *Histology for Medical Students*, Oxford Medical Publications.

Glassware—To Clean.**(i) Slides and cover-slips.**

1° Soak in

either (a) Old xylene 1 part.

Methylated spirits 1 part.

or (b) Sodium hydroxide (conc.
aq.).

or (c) Nitric acid (conc.).

or (d) Sulphuric acid (conc.), or
nitric acid (conc.) 1 part.
Potassium dichromate
(3% aq.) 9 parts.

2° Wash in running tap-water.

3° Rinse in distilled water.

4° Rinse (store if desired) in acid alcohol.

5° Dip in alcohol (90%).

6° Wipe dry on a *dry, clean, lint-free* cloth.**(ii) To remove grease.**

Either, (a) Soak in

Sulphuric acid (conc.) satis.

Potassium dichromate excess.

or, (b) wash with "Dreft" [Trade name for
one of the sulphated fatty alcohols (S.F.A.'s)].Use 5% solution with a little ammonium hydroxide
added.*Subsequent treatment after (a) and (b) as (i) 2° and
3°, and, if for microscopic work, 4°–6° above.**(iii) To remove white deposit.**

Soak in sodium metasilicate (5% aq.).

(iv) Blood pipettes.Attach by pressure tubing to a filter pump and dip
the ends successively in distilled water, alcohol
(100%), and ether.**(v) Microscope lenses. See p. 57.**

* Recommended by J. W. Davis, *School Science Review*, XX, 80, 619.
June 1939.

Glassware—To Write On. See "Grease Pencil" (p. 251) and "Ink for writing on Glass" (p. 255).

Glycerine.

Glycerine	50 c.c.
Distilled water	50 c.c.
Thymol (conc. soln.)	1 c.c.

Glycerine-Albumen. See "Egg Albumen" (p. 243).

Glycerine Dextrin.* (Adhesive for diatom mounting.)

Dissolve dextrin in water till it is almost a jelly. Add glycerine and shake thoroughly until as much dextrin as possible has been dissolved in the glycerine. Add a few drops of phenol.

Glycerine Jelly.

Gelatine	1 part by weight.
Distilled water	6 parts by weight.

Leave for 2 hours. Add:

Glycerine	7 parts by weight.
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Add, for every 100 grams of mixture,

Thymol (pure)	1 gr.
---------------	-------

Warm and stir for 15 mins. until flakes produced by the thymol have disappeared.

(The use of *thymol*—instead of phenol—is advantageous because phenol tends to cause fading of stains and of chlorophyll.)

For method of use, refer to "Mountants" II (ii) (p. 45).

Golden Syrup.

For use as an immersion medium for high-power lenses, dilute it with an equal volume of distilled water.

Gold Size.

If necessary to dilute, do so with linseed oil.

Gold size is soluble in oil of turpentine.

* Reproduced by courtesy of Messrs. W. Watson & Sons, Ltd.

Gossypimine. See "Safranin O" (p. 280).

Gram's Iodine.

Bacteria ; Nuclei (plant) (counterstain with crystal violet and orange G in clove oil).

Iodine	1 gr.
Potassium iodide	2 gr.
Distilled water	300 c.c.

Gray's Sealing Medium. See "Ringing Media" (iii) (p. 279).

Grease Pencil (Foertsch's)* (For writing on glass.) [See also "Ink for writing on Glass" (p. 255).]

White wax	8 parts.
Tallow	2 parts.

Melt together and stir in Prussian blue till required intensity of colour is obtained. Cool. When nearly cold, roll into pencils of suitable size on a slab. Cover with a paper case.

Grease—To Remove. See "Glassware—To Clean" (p. 249).

Grenacher's Alcoholic Borax Carmine. See "Borax Carmine" (p. 230).

Gum Arabic.

(i) See "Gum for Labels" (p. 252), and "Adhesive" (p. 222).

(ii) *To slacken movements of spermatozooids.*
10% aq. filtered through swansdown.

Gum Dammar.

Dammar resin.

Xylene.

Dissolve dammar resin in xylene until a thin solution is obtained. Filter through filter paper wetted with chloroform.

* After Geoffrey Martin (Ed.), *Dyestuffs and Coal Tar Products*, Crosby Lockwood.

Gum for Labels.*(i) *Solution (a) :*

Gum tragacanth	30 gr.
Water	250 c.c.

Stand for some hours. Shake until the liquid froths, and mix with :

Solution (b) :

Gum arabic	120 gr.
Water	250 c.c.

Strain the mixture through linen and add :

Mixture (c) :

Glycerine	150 gr.
Oil of thyme	2.5 gr.

- (ii) See also "Adhesive for Glass" (p. 222) and "Cellophane—Gum for" (p. 234).

Hæmatoxylin. C.I. No. 1246.

The colour resulting from hæmatoxylin depends upon the mordant used. Potash alum gives purple-blue. Iron alum gives black. See Ammonia-Hæmatin; Anderson's Iron-Alum Hæmatoxylin; Carazzi's Hæmatoxylin; Delafield's Hæmatoxylin; Ehrlich's Hæmatoxylin; Heidenhain's Hæmatoxylin; Mayer's Acid Hæmalum; Renaut's Eosin-Hæmatoxylin.

Hæmatoxylin-Eosin. See "Renaut's Eosin-Hæmatoxylin" (p. 278).

Hanstein's Rosaniline (Fuchsin) Violet.†

Chlorophyll cell-wall stratification; Stem-sections (esp. monocots.); Amyloid substances, Nucleus, Gums (→ red shades); Protoplasm (→ bluish violet); Resins (→ blue); Tannin (→ foxy-red); Cellulose (→ pale violet); Lignin (→ reddish); Bast-fibres (→ deep

* After Lee, *Microtomist's Vade-Mecum*, Churchill.

† After Strasburger, *Handbook of Practical Botany* (English edn.), Allen and Unwin.

red); Sieve-tubes and bast parenchyma (→ hardly stained); Zoophytes.

Methyl violet 2B (p. 265) 1 part.
 Basic fuchsin (p. 228) 1 part.
 Alcohol (70%) To give a saturated solution.

Heidenhain's Hæmatoxylin. (With iron alum mordant.)

Nuclear. A useful stain. Cell walls (→ light blue); Cytology; Mitotic figures (→ black); Nuclei (plant and animal) (→ dark blue); Skin, Thymus, and Suprarenal body (all counterstained with van Gieson's); Algæ (mordant with liquor ferri).

Hæmatoxylin 1 gr.
 Alcohol (100%) 100 c.c.
 Distilled water 90 c.c.

The hæmatoxylin may be differentiated with iron alum.
 See "Iron Alum" (ii) (p. 256).

Hoffmann's Blue (Acetic-aniline blue).

Callose of sieve plates; Protoplasmic cell contents (washes out of cell walls with water or glycerine).

Aniline blue W.S. (see p. 226) 1 gr.
 Acetic acid (glacial) 1 c.c.
 Alcohol (50%) 98 c.c.

Before staining, wash alcohol-preserved material in water. After staining, wash in water and mount in glycerine.

Hofmann's Grün. See "Iodine Green" (p. 256).

Hofmann's W.S. Violet. (Dahlia.) C.I. No. 679.

Nuclear. Fresh animal tissue; *Lumbricus* (seminal vesicles); Nuclei (plant); Protozoa; Spermatozoa.

Hofmann's W.S. violet 0.25 gr.
 Distilled water 100 c.c.

Hydrogen Peroxide.

For bleaching after fixation with osmium tetroxide.

Hydrogen peroxide (20 vols. soln.)	40 c.c.
Alcohol (60%) or (80%)	60 c.c.

Hydroquinone.

Reducing fluid after treatment with silver nitrate.

- (i) For use in Cajal's method for Golgi bodies.

Hydroquinone	2 gr.
Formaldehyde (40%)	15 c.c.
Sodium sulphite (anhydrous)	0.15 to 0.5 gr.
Distilled water	100 c.c.

- (ii) For use with mitochondria.

(Modification of Cajal's method for Golgi bodies.)

Hydroquinone	2 gr.
Formaldehyde (40%)	20 c.c.
Distilled water	100 c.c.

Hypo. See "Sodium Thiosulphate" (p. 284).

Injection Fluid.

- (i) *Guyer's.*

Carmine	5 gr.
Water.	10-12 c.c.

Grind up carmine in the water and add drops of ammonium hydroxide until the solution becomes transparent. Soak 50 gr. gelatine (best sheet) in cold water (24 hours). Pour off water and melt gelatine at 40° C. Pour in colouring matter. Stir. While cooling add drops of acetic acid (25% aq.) till the mass is opaque and smells faintly acid.

If a blue colour is desired, add Aniline Blue W.S. (instead of carmine) to the molten gelatin.

- (ii) Gelatine (powdered)
- | | |
|-----------------|----------|
| Distilled water | 10 gr. |
| | 200 c.c. |

Dissolve the gelatine in the distilled water. Then add Lead acetate

	50 gr.
--	--------

When dissolved add gradually Potassium chromate (powdered)

	25 gr.
--	--------

- (iii) Gelatine 10 gr.
 Chloral hydrate 10 gr.
 Water 100 c.c.

If the gelatine is in sheets, use a jacketed heater to assist solution. Otherwise, use powdered gelatine.

When solution is complete, add:

Chrome yellow (powdered), till the desired depth of colour is obtained.

The chloral hydrate delays setting of the gelatine.

Once the gelatine has set, the action is irreversible.

These injection masses must be kept warm during injection.

Ink for Writing on Glass. [See also "Grease Pencil" (p. 25).]

- (i) Shellac [13% soln. in cold alcohol (96%)] 30 c.c.
 Borax [13% soln. in distilled water] . . 50 c.c.

Mix the two solutions a drop at a time. If necessary, heat gently to dissolve any precipitate.

When solution is complete add:

Methylene blue or other dye—sufficient to give the required depth of colour.

- (ii) Gurr's glass ink.

Iodine.

A temporary stain for:

Cellulose (→ yellow) or, + sulphuric acid (40%) (→ blue); *Chara* (with basic fuchsin); Glycogen; Hyphæ (protoplasm) (with basic fuchsin); *Nitella* (with basic fuchsin); Protein cell contents in plant tissue (→ brown); *Protococcus* (with basic fuchsin); Protoplasm (→ brown); Starch (→ blue); *Vorticella* (with basic fuchsin).

- (a) Potassium iodide satd. aq. soln.
 add

Iodine to saturation.

Filter. Dilute with distilled water to a sherry colour.

(b) *Formula for use in Breinl's stain.*

Iodine	1 gr.
Potassium iodide	1 gr.
Alcohol (80%)	100 c.c.

(c) See also Gram's iodine (p. 251), Lugol's iodine (p. 261), and Schulze's solution (p. 282).

Iodine for Washing Tissue fixed with Mercuric Chloride.

Potassium iodide	3 gr.
Iodine	2 gr.
Alcohol (70%) or (96%) (as required)	100 c.c.

Iodine Green (Hofmann's Grün). C.I. No. 686.

Plasma. Lignin (counterstain with acid fuchsin, Mayer's carmalum, eosin Y, erythrosin bluish, or magdala red).

(1) Iodine green	1 gr.
Alcohol (70%) (or distilled water)	100 c.c.

(2) See also "Acetic-Iodine Green" (p. 220).

Iron-Aceto-Carmine.

Stain the material in aceto-carmine (p. 221) on the slide and stir it with a *steel* teasing needle.

Irish Moss. See "Carragheen Solution" (p. 234).

Iron Alum.

- (i) $\frac{1}{2}\%$ aq.
- (ii) *Mordant and differentiator for Heidenhain's hæmatoxylin and Anderson's Iron-Alum-Hæmatoxylin.*
2% aq.

Iron Hæmatoxylin. See "Anderson's Iron-Alum-Hæmatoxylin" (p. 226), "Heidenhain's Hæmatoxylin" (p. 253) and "Weigert's Iron Hæmatoxylin" (p. 287).

Iron alum can be used to differentiate iron hæmatoxylin.

Isotonic Saline. See "Saline Solutions" (p. 281).

Janus Green B. C.I. No. 133.

Nuclear and Plasma. (A basic dye.) Living organisms.

Janus green B 0.1 gr.

Isotonic saline 1,000 c.c.

Jaune Metanil. See "Metanil Yellow" (p. 265).

Kaiserling-Gelatin.*

1° Heat to boiling point in a suitable vessel (stirring to prevent burning):

Kaiserling's solution (p. 258) . . 3,000 c.c.

Gelatin 200 gr.

2° When the temperature reaches 55° C. stir in:

The whites of three eggs.

Acetic acid, sufficient to make strongly acid to litmus paper.

(This helps clarification and subsequent colour conservation.)

3° Allow to boil for a *few minutes*.

4° Filter through two thicknesses of paper into stock jars. (Use ribbed funnels and a hot-water filter, or fix the whole in a steam sterilizer to prevent solidification of jelly.)

5° If the jelly is required for stock purposes, after it has set place a crystal of thymol on the top of the jelly (to prevent the growth of fungi) and close the vessel.

6° Before use for mounting, remove the thymol crystal.

7° Melt jelly (45° C.-50° C.) by placing the container on a water-bath.

8° To every 1,000 c.c. of melted jelly stir in:

Formaldehyde (40%) . . . 27.5 c.c.

The jelly is now ready for use.

* Adapted from information supplied by the Pathological Department, Cheltenham General Hospital.

Kaiserling's Solution.*

Potassium acetate	100 gr.
Glycerine	200 c.c.
Water	1,000 c.c.

Killing Animals. See Chapter VI (p. 111), "Methods for Specific Material," under animal required.

Killing Bottle.(i) *For terrestrial arthropods.*

Potassium cyanide (*Poison*)—1 oz. Break up the cyanide and place it in a 10 oz. wide-mouthed bottle with an air-tight bung. Plaster-of-Paris—Mix sufficient with water to a thickness that will just allow of its running from the mixing vessel and covering the cyanide to a depth of from $1\frac{1}{2}$ " to 2". Soon sets hard and dry if properly mixed.

(ii) *For most aerial arthropods. (Except humble-bees and larger beetles.)*

Cut laurel leaves into narrow strips and pack them fairly tightly in a tin or wide-mouthed bottle. Cover with muslin.

(iii) *For mosquitoes, flies, bees.*

In the bottom of a specimen tube (3" × 1") place a wad of cotton-wool covered with a piece of perforated zinc cut to size. Damp the cotton-wool with ethyl acetate [or, in an emergency, petrol, benzene, xylene (care), or chloroform]. See that the cork is a good fit. The ethyl acetate keeps the muscles relaxed, and insects may be stored in this liquid. If carried in the pocket, it is well to provide this killing tube with a protective case.

Standing insects (e.g. mosquitoes) may be caught by inverting the open tube over them.

(iv) *For relaxing animals killed with cyanide.*

Used chopped laurel leaves.

* Adapted from information supplied by the Pathological Department, Cheltenham General Hospital.

Knop's Solution.

For culture of algæ.

Solution (a)	Magnesium sulphate	1 gr.
	Potassium nitrate	1 gr.
	Potassium phosphate	1 gr.
	Dissolve in distilled water.	1,000 c.c.
Solution (b)	Calcium nitrate	4 gr.
	Dissolve in distilled water.	1,000 c.c.

Add solution (b) to solution (a).

Kronig's Deckglaskitt. See " Ringing Media " (ii) (p. 279).

Labels.

Bottles and tubes of plant and animal specimens should bear a label *inside* as well as outside.

- (i) *Inside labels.* Use plain white paper, write in pencil, and state, not only the name of the tissue, but also the treatment received up to the time of bottling and the name and strength of the preservative. A knowledge of such information may influence the course of future treatment.
- (ii) *Outside labels.* Use good quality paper and good quality gum. Write legibly in Indian ink. After fixing label to bottle, brush over with a thin layer of molten paraffin wax if the specimen is likely to be kept for a long time. The wax covering is effective against an over-dry or an over-damp atmosphere.
- (iii) See " Gum for Labels " (p. 252); " Ink for Writing on Glass " (p. 255); and " Grease Pencil " (p. 251).

Lacto-Phenol.

Lactic acid (conc.)	10 gr.
Phenol (crystals)	10 gr.
Glycerine	20 gr.

or, when using Godwin's method for

fungi, (iv) (c) (2) (p. 163)	10 gr.
Distilled water	10 c.c.

Keep in a brown glass bottle. Permanent preparations must be ringed.

Laurel. See "Killing Bottle" (ii) (p. 258).

Leishman's Stain.

Blood and blood parasites. For results of staining, see "Blood" (iv) (c) (p. 135).

Preparation of the dyestuff is difficult. Buy it ready made.

Leishman's stain	0.15 gr.
Methyl alcohol (100%) (free from acetone)	100 c.c.

Leuco-Malachite Reagent.

For blood stains. See "Blood" (v), p. 136.

Solution A.

Leuco-malachite green	1 gr.
Acetic acid (glacial)	100 c.c.
Distilled water	150 c.c.

Keep in the dark, with a paraffin-wax cover over the stopper.

Solution B.

Hydrogen peroxide (1%).

For use in blood-stain tests mix 8 c.c. of solution A with 2 c.c. of solution B. Before using on the unknown stain [see "Blood" (v) (p. 136)] test the reagent on an old blood smear.

Light Green S.F. Yellowish (Acid green). C.I. No. 670.

Plasma. (An acid dye.) Plant tissue (counterstain with hæmatoxylin or safranin O). Cellulose (→ green); cell walls (unlignified) (→ green); cytoplasm (→ green); *Paramecium* (nuclear detail).

(a) Saturated solution in alcohol (90%).

(b) *For plant mitosis* (see "Mitotic Figures" (iii) (d), p. 180).

Saturated solution in alcohol (95%).

(c) *In clove oil* (see "Plant Histology," II (a), p. 195).

Light green S.F. yellowish (powdered)	0.2 gr.
Alcohol (100%)	50 c.c.
Clove oil	50 c.c.

Liquor Ferri.*

Used as a mordant for Heidenhain's hæmatoxylin, especially when staining algæ.

Ferrous sulphate	8.5 gr.
Distilled water	4.5 c.c.
Nitric acid	1.8 c.c.
Sulphuric acid	1.5 c.c.

Dilute each c.c. of this solution with 25 c.c. of distilled water to make the stock solution.

For use, dilute this stock solution to a very pale straw colour with distilled water.

Lithium Carbonate.

For removal of picric acid from sections. (2 mins.).

Alcohol (70%)	100 c.c.
Lithium carbonate	To saturation.

Loeffler's Methylene Blue.

Nuclear. Bacteria; Lignin (counterstain with eosin Y or erythrosin bluish); Nuclei.

Solution A.

Methylene blue (see p. 265)	0.3 gr.
Alcohol (95%)	30 c.c.

Solution B.

Potassium hydroxide (0.01% aq.)	100 c.c.
Mix solutions A and B. (The potassium hydroxide is an accentuator.)		

Lugol's Iodine Solution.

Iodine	4 gr.
Potassium iodide	6 gr.
Distilled water	100 c.c.

* With acknowledgments to C. F. Bause.

Magdala Red. C.I. No. 857.

(Naphthalene Red; Naphthalene Pink; Naphthylamine Pink; Sudan Red.)

(A basic dye.) Algæ (with aniline blue W.S.); Cellulose; Elastin; Mosses (with aniline blue W.S.); Nuclei.

(a) Magdala red	2.0 gr.
Distilled water	98 c.c.

(b) *For algæ*:

Magdala red	0.2 gr.
Alcohol (95%)	100 c.c.

Magenta. See "Basic fuchsin" (p. 228).

Malachite Green. C.I. No. 657.

Nuclear. (Weakly basic.) Lignin.

(a) Malachite green	1.0 gr.
Distilled water	99 c.c.

(b) See also "Pianese IIIb stain" (p. 271).

Mallory's Triple Stain.*

Nuclear and Plasma. *Ascaris*; Cartilage; Connective tissue (→ blue); Cytoplasm (→ red); Elastic fibres are unstained; Erythrocytes (→ red); *Fasciola*; *Hirudo*; Intestine; Lymph glands; Neuroglia (→ red); Nuclei (→ red); Ovary; Pancreas; Pituitary; Salivary glands; *Taenia*; Testis; Thymus; Thyroid. A triple stain giving good results for routine work after fixation in Zenker's solution.

For method of use see Method PW-1 (pp. 98-106).

Solution A.

Acid fuchsin (see p. 222)	0.1 gr.
Distilled water	100 c.c.

Solution B. Phosphomolybdic acid (1%) (the mordant).

* After Green, *School Science Review*, XV, 406; March 1934.

Solution C.

Aniline blue W.S. (see p. 226)	0.5 gr.
Orange G (see p. 268)	2.0 gr.
Oxalic acid	2 gr.
Distilled water	100 c.c.

Mann's Fixative.*Stock solution.*

Picric acid	1.0 gr.
Mercuric chloride	2.5 gr.
Distilled water	To make up to 100 c.c.

Before use, add

Formaldehyde (40%), 15 c.c. to every 100 c.c. of stock solution.

Mann's Stain.

A good double stain for sections.

Methyl blue (1% aq.) (see p. 265)	35 c.c.
Eosin Y (1% aq.) (see p. 244)	35 c.c.
Distilled water	100 c.c.

Martius Yellow. C.I. No. 9.

An acid dye.

For use, see "Pianese IIIb Stain" (p. 271).

Mayer's Acid Hæmalum.*

Nuclear. Bulk and section staining of animal tissue.

Small entire animals; Nuclei.

Hæmatoxylin	1 gr.
Distilled water	1,000 c.c.
Dissolve, and add sodium iodate	0.2 gr.
Potash alum	50 gr.
Dissolve, and add glacial acetic acid	20 c.c.
Chloral hydrate	50 gr.
Citric acid	1 gr.
Phenol	1 crystal.

* For routine purposes J. R. Baker (*Science Progress*, XXXI, 121, 189; July 1936) recommends Mayer's Acid Hæmalum in preference to Delafield's hæmatoxylin (unstable) and Ehrlich's hæmatoxylin (slow) and all other hæmatoxylin and hæmatins.

Mayer's Alum Carmine.

Nuclear. (An acid dye.) Small entire animals (not marine). (May be followed by picric acid); *Cladophora* sp.

Carmine	2 gr.
Alum	5 gr.
Distilled water	100 c.c.

Boil for an hour.

Mayer's Carmalum.

Cellulose (counterstain with iodine green); Cell contents; Small entire animals (not marine); Unlignified tissue.

Carminic acid	1 gr.
Alum	10 gr.
Distilled water	200 c.c.

Dissolve (heat if necessary). Decant or filter, add an antiseptic (e.g. thymol or 0.10% salicylic acid).

Mayer's Egg-Albumen. See "Egg Albumen" (p. 243).

Mayer's Mucicarmine.*

Stock solution.

Carmine	1 gr.
Aluminium hydroxide (powdered)	1 gr.
Aluminium chloride (anhydrous— freshly powdered in mortar)	0.5 gr.
Alcohol (50%)	100 c.c.

Boil on a water-bath. Shake frequently (2½ mins.).

Cool quickly. Filter cold.

For use, take

Stock solution	1 c.c.
Distilled water	9 c.c.

Diluted solution will keep for 2 days.

Counterstain with metanil yellow (p. 265).

Mercuric Chloride.

For fixation use a saturated aqueous solution.

* Southgate, *Journal of Pathology and Bacteriology*, 30, 729; 1927.

Metanil Yellow. (Jaune Metanil.) C.I. No. 138.

Counterstain for Mayer's mucicarmine.

Metanil yellow	1 gr.
Distilled water	100 c.c.

Methyl Blue. C.I. No. 706.

(An acid dye.) A good counterstain for carmine, eosin Y, picric acid, and safranin O.

- (a) Methyl blue 1.0 gr.
Distilled water 99 c.c.
- (b) See "Mann's stain" (p. 263).

Methyl Green. C.I. No. 684.

Nuclear. (A basic dye.) Bast (→ blue); Fresh tissue (temp.); Lignin (→ green); Living organisms; Nuclei (→ green); Protozoa; Small entire objects (temp.); *Vorticella*. May be counterstained with acid fuchsin.

- (a) Methyl green 1 gr.
Alcohol 99 c.c.
- (b) See also "Acetic-Methyl Green" (p. 221).

Methyl Violet 2B. C.I. No. 680.

Nuclear. Gives a reddish shade (cf. Crystal violet, p. 238). Amyloid (animal) (→ reddish); Bacteria; Blood.

Alcoholic (or Aqueous) soln.

Methyl violet 2B	1 gr.
Alcohol (70%) (or distilled water)	99 c.c.

Do not use any alcohol after using the aqueous soln.

Methylene Blue (and Methylene Blue B). C.I. No. 922.

Nuclear. (A basic dye.) Bacteria; Blood; Epithelia; Mucin (→ purple); Nerve tissue (nuclei, axons, dendrons in spinal cord); Protozoa; Yeast. For living organisms, use a solution in saline. Counterstain for

Ziehl's carbolic-fuchsin and Ehrlich's aniline-water fuchsin.

- (a) Methylene blue 0.3 gr.
 Alcohol (95%) 30 c.c.
 Dissolve, and add
 Distilled water 100 c.c.
- (b) *For living organisms.*
 Methylene blue 1 gr.
 Sodium chloride 0.6 gr.
 Distilled water 100 c.c.
- (c) See also acetic methylene blue, Borrel's methylene blue, carbol methylene blue, Nissl's methylene blue, Loeffler's methylene blue, xylene-methylene blue.
- (d) Methylene blue is not to be confused with New Methylene Blue N (p. 267), or with Polychrome Methylene Blue (p. 273).

Microscope, Cover for.

When it is desired to leave a microscope out of its normal case, or cupboard, or both, for a short time, it is useful to have a cover made of "Cellophane" of ample size and suitable shape.

15% of glycerine added to gum arabic, or to any good glue or gum, or to waterglass, gives a suitable adhesive for "Cellophane."

"Milton."

For differentiating chlorazol black E, use a dilute soln. *Note.* "Milton" is a trade mark, but no proprietary rights are claimed in the preparation itself:

Sodium hypochlorite	1.00%
Sodium chloride	16.50%
Sodium chlorate	0.13%
Sodium carbonate	0.05%
Sodium sulphate	0.15%
Calcium chloride	0.07%
Magnesium chloride	Trace.
Water	82.10%

Moll's Solution.

For clearing, in Dawson's method for bone (p. 137).

Glycerine	20 c.c.
Potassium hydroxide (1%)	80 c.c.

Müller's Fluid.

For hardening; dissolving intercellular substances prior to teasing; preserving brains; and fixation of nervous tissue and eye.

Potassium dichromate	25 gr.
Sodium sulphate	10 gr.
Distilled water	1,000 c.c.

Naphthalene Pink. See "Magdala Red" (p. 262).

Naphthalene Red. See "Magdala Red" (p. 262).

Naphthylamine Pink. See "Magdala Red" (p. 262).

Neutral Red. C.I. No. 825.

Nuclear and Plasma. (A mildly basic, non-toxic dye.)
Living organisms; Nuclei (→ red); Plasma (→ yellow); Food vacuoles of living *Paramecium*.

Neutral red	0.1 gr.
Isotonic saline	1,000 c.c.

Newton's Gentian Violet. See under "Crystal violet" (a) (p. 238).

New Methylene Blue N. C.I. No. 927.

For van Wijhe's method for cartilage (p. 140).

New methylene blue N	0.25 gr.
Alcohol (70%)	100.0 c.c.
Hydrochloric acid (conc.)	1.0 c.c.

Nigrosin, Water Soluble. C.I. No. 865.

<i>Plasma.</i> Cell contents; <i>Obelia</i> ; Unlignified tissue.		
Nigrosin, water soluble	.	1 gr.
Distilled water	.	100 c.c.

Nile Blue Sulphate. C.I. No. 913. (A basic dye.)

Living amphibian eggs, *Hydra*, protozoa, yeast.

(a) Nile blue sulphate	.	0.1 gr.
Distilled water	.	100 c.c.
(b) See also "Xylene-Methylene Blue" (p. 289).		

Nissl's Methylene Blue.*

Nerve tissue (nuclei, axons, dendrons in spinal cord).

Use after fixation in alcohol (95%) without subsequent hydration.†

Venetian soap	.	1.75 gr.
Distilled water	.	1,000 c.c.

Dissolve, and add:

Methylene blue B	.	3.75 gr.
------------------	---	----------

Shake vigorously from time to time and do not use *till four months old*. Filter into the stock bottle any of this stain left after staining sections.

Orange Fuchsin.

Plasma. Cytoplasm; Connective tissue.

Orange G (see below)	.	6 gr.
Acid fuchsin (see p. 222)	.	1 gr.
Alcohol (100%)	.	60 c.c.
Distilled water	.	240 c.c.

Orange G. C.I. No. 27.

Plasma. (An acid dye.) Cellulose (→ yellow); Nuclei (counterstain to Gram's iodine and crystal violet); *Vorticella* (with iron hæmatoxylin).

(a) Satd. soln. aq.

* After Lee, *Microtomist's Vade-Mecum*, Churchill.

† See "Nerve Tissue" (iv), p. 187.

(b) *In clove oil:*

Orange G 1 gr.

Alcohol (100%) 100 c.c.

Dissolve the orange G in the alcohol. Add:

Clove oil 100 c.c.

Allow the alcohol to evaporate till 100 c.c. of solution remains.

(c) See also Mallory's triple stain (p. 262) and orange fuchsin (p. 268).

Orange Tannin.

This is a mixture of orange G and tannic acid.

For use in Breinl's stain, use as supplied by British Drug Houses, Ltd., Graham Street, City Road, London, N.1.

Orcein (and Orcein D). C.I. No. 1242.Elastic fibres [nuclei (\rightarrow blue); cytoplasm (\rightarrow pink)];
[do not differentiate with acid alcohol]; Inulin (\rightarrow orange-red).(a) *Alcoholic soln.*

Orcein 1 gr.

Alcohol (100%) 100 c.c.

Hydrochloric acid (conc.) 1 c.c.

(b) *Aqueous soln.*

Orcein 2 gr.

Acetic acid (glacial) 2 c.c.

Distilled water 100 c.c.

"Osmic Acid." See "Osmium Tetroxide" (below).

Osmium Tetroxide.Fats; Lipoids (\rightarrow brown).(i) *For fixing protozoa.* 0.25% aq.(ii) *For fixing animal tissue for cytological purposes.*
1% aq.(iii) *For staining fats, etc., and for fixing plant tissue for histological purposes.* 2% aq.

- (iv) Should be kept in an uncoloured glass bottle (to enable contents to be seen), but this *must* be enclosed in a suitable light-tight box and kept away from the light. One drop of mercuric chloride (satd. aq.) is said to prevent the reduction of 10 c.c. of osmium tetroxide (2%).* Keep away from fingers, eyes, and mucous membrane.

Pampel's Fluid.

For preservation of biological specimens, especially insects.
See "Animal Tissue" (iii) (p. 313).

Acetic acid (glacial)	.	.	.	4 volumes.
Formaldehyde (40%)	.	.	.	30 "
Alcohol (95%)	.	.	.	6 "
Distilled water	.	.	.	15 "

Paraffin Wax. *For embedding and impregnating.* Melting point 48° C.-53° C. (not higher).

Pasteur's Solution.

Cane sugar	.	.	.	15.0 gr.
Ammonium tartrate	.	.	.	1.0 gr.
Dipotassium hydrogen phosphate	.	.	.	0.2 gr.
Calcium phosphate	.	.	.	0.02 gr.
Magnesium phosphate	.	.	.	0.02 gr.
Distilled water	.	.	.	100 c.c.

Perenyi's Fluid.

For preserving frog spawn.

Nitric acid (60%)	.	.	.	4 c.c.
Alcohol (90%)	.	.	.	18 c.c.
Chromic acid	.	.	.	0.09 gr.
Distilled water	.	.	.	38 c.c.
Jelly dissolved in a few weeks.				

Phenol-Turpentine. See "Carbol-Turpentine" (p. 233).

* Baker, *Cytological Technique*, Methuen.

Phloroglucin(ol) (Symmetrical trihydroxybenzene).

Lignin (temp.) (soak tissues and add conc. hydrochloric acid) (→ violet-red) (this is a specific stain for lignin);
 Inulin (+ dil. hydrochloric acid) (→ orange-red).

Phloroglucin(ol)	10 gr.
Alcohol (100%)	95 c.c.

Phloxine B. C.I. No. 778.

(An acid dye.) Algæ. Counterstain to aniline blue W.S.
 (*Chamberlain's method*, p. 112.)

Phloxine B	1 gr.
Alcohol (90%)	99 c.c.

Pianese IIIb Stain.

Fungal mycelium in host plant (p. 163).

Martius yellow (p. 263)	0.01 gr.
Malachite green (p. 262)	0.50 gr.
Acid fuchsin (p. 222)	0.10 gr.
Alcohol (95%)	50 c.c.
Distilled water	150 c.c.

Picric Acid. C.I. No. 7.

Plasma. Chitin (→ yellow); Erythrocytes; Hair;
 Horn; Lignified tissue; *Nereis*—parapodia (preceded
 by borax carmine); Nerve tissue (in dissections);
 Trachea of insect (preceded by borax carmine);
 Muscle; may be preceded by hæmatoxylin.

(a) *Alcoholic soln.*:

Alcohol (100%) with picric acid to saturation.
 Decant.

(b) *Aqueous soln.*:

Distilled water with picric acid to saturation.
 Decant.

(c) *Picric-alcohol*:

Alcohol (50%) with picric acid to saturation.
 Decant.

(d) *Picro-clove oil* :

Clove oil with picric acid to saturation. Decant.

(e) See also picric-aniline blue W.S. and picro-carmin (below).

Picric-Alcohol. See "Picric Acid" (c) (p. 271).

Picric-Aniline-Blue W.S.

Counterstain to safranin O. Chromatin in fresh material (use with safranin O); Fresh animal tissue.

Aniline blue W.S. (p. 226)	2 gr.
Picric acid (p. 271)	1 gr.
Alcohol (80%)	1,000 c.c.

Picro-Carmine.

Nuclear. (A neutral stain.) A good double stain for general animal histology, especially for sections of nervous tissue. Differentiate in acid water. Mount in Farrants' medium. Stains best with tissue preserved in alcohol (70%). Nuclei (→ red); Cytoplasm (→ yellow).

Carmine (p. 233)	10 gr.
Ammonium hydroxide (Sp. Gr. 0.880)	40 c.c.
Distilled water	2,000 c.c.
Dissolve, and add :	
Picric acid (p. 271)	50 gr.

Shake well for a few minutes, allow to stand; decant.

Allow to stand a few days, stirring occasionally.

Evaporate to dryness over a water-bath.

To make up the stain :

Above residue	2 gr.
Distilled water	100 c.c.

Picro-Clove Oil.

Chitin (→ yellow or brown) (preceded by borax carmine);

Crustacea (preceded by borax carmine).

Clove oil with picric acid to saturation.

Picro-Formo-Acetic. See "Bouin's Fluid" (p. 231).

Pith. See "Elder Pith" (p. 244).

Polychrome Methylene Blue.

This dye is *not* identical with methylene blue C.I. 922 ; it is a mixture of dyes obtained by the alkaline oxidation of methylene blue.

Formula for use in Breinl's stain.

Polychrome methylene blue	.	.	.	7	gr.
Sodium carbonate	.	.	.	0.5	gr.
Distilled water	.	.	.	100	c.c.

Improves by keeping.

Potash Alum.

For dilution of old Ehrlich's haematoxylin. Satd. aq. soln.

Potassium Acetate.

Potassium acetate satd. aq. soln.

Potassium Dichromate.

For fixation of animal tissue for cytological purposes.
1½% aq. soln.

Potassium Ferrocyanide.

For staining iron compounds in sections.

Use a fresh mixture of equal parts of

Potassium ferrocyanide	.	.	.	1.5%	aq.
Hydrochloric acid	.	.	.	0.5%	aq.

Potassium Hydroxide.

To remove fats and roughen cuticle of nematodes. 5% aq.

Potato Agar.

<i>Peeled</i> potatoes	200 gr. (approx. 7 oz.).
Water	1,000 c.c.
Cut the peeled potatoes fine, and boil for 15-20 <i>mins.</i>	
Strain off the liquid,	
Make up with water to	1,000 c.c.
Add:	
Agar-agar	15 gr.

Boil until dissolved, filter, pour about 10 c.c. into each tube. Sterilize in a steamer for 1 *hour* on *three successive days*. Do not remove the lid in the meantime. If "slanted" tubes are required, allow to solidify in a reclining position.

Preserving Fluids.

Only named fluids are listed in this chapter.

Un-named fluids will be found (listed under the name of the organism or tissue for which they are to be used) in Chapter IX, p. 312.

Prune Agar.

For culture of fungi.

Prunes	about 25.
Cane-sugar	400 gr.
Agar-agar	50 gr.

Boil the prunes in about 500 c.c. of water (1 *hour*). Decant and make up to 1,000 c.c. Add the cane-sugar and the agar.

Quinoline Blue. See "Cyanin" (p. 239).

Raisin Gelatin.

Raisins 30 gr. (approx. 1 oz.)
Distilled water 1,000 c.c.
Cut up the raisins, boil for 15 mins. Strain off raisins.
Add,
Gelatin 120 gr. (approx. 4½ oz.)
to the extract. Heat to dissolve gelatin.
Filter through paper, pour into tubes [see "Potato
Agar" (p. 274)].
Sterilize for 30 mins. in a steamer on three successive
days.

Razor, to Sharpen.*

- (i) *Grinding*. This should never be attempted by amateurs.
- (ii) *Honing*. This becomes necessary when the keen, angular edge of the razor has become rounded by constant stropping, or when a feather-edge remains after grinding.
 - 1° Use a Turkey stone. Thinly cover the working surface of the stone with olive oil.
 - 2° Wedge a piece of folded paper between shank of razor and handle to keep the blade steady.
 - 3° Sit at a table with the length of the stone arranged parallel with the edge of the table, in front of the body.
 - 4° Place the razor blade *flat* on the left end of the stone with its edge pointing to the right and with its heel (i.e. the end nearer the handle) rather in advance of its toe (i.e. "heel-to-toe").
 - 5° With gentle pressure pull the razor to the right, *edge first*, "heel-to-toe."
 - 6° At the end of the stroke turn the razor over, **ON ITS BACK**, and draw it to the left, *edge first*, "heel-to-toe."

* I am indebted to Mr. W. J. Clare, of Messrs. R. A. Lister & Co., Ltd., Dursley, for these instructions.

7° Gradually decrease the pressure and shorten the stroke.

8° It may be found necessary, finally, to tilt the razor on to its toe, and, with a circular motion, rub off the toe.

Note: 1. Both wedge-shaped and hollow-ground (plano-concave) blades are honed in the same manner.

2. Keep the Turkey stone covered and free from dust, and remove surplus oil after use.

(iii) *Stropping.* Razors should always be stropped immediately before and after use.

1° Before use, wipe the strop with the palm of the hand to remove any adhering dust or grit.

2° With one hand hold the strop taut, and with the other press the razor blade *flat* on the strop.

3° Draw the blade along the strop, away from the body, *back first*, "heel-to-toe." [See (ii) 4° p. 275.]

4° At the end of the stroke turn the razor over, ON ITS BACK, and draw it, *back first*, "heel-to-toe," towards the body.

5° Give about 8 strokes on the rough, followed by about 10 on the smooth side of the strop.

6° Finally, using the palm of the hand as a strop, give about 8 strokes as directed in 3° and 4°.

(iv) *General Care.*

1. Always strop the razor before use.

2. Never lay the razor, with blade exposed, on the bench.

3. After use, clean the blade with xylene and draw it, edge first, away from the body, in the folds of a soft duster held between finger and thumb.

4. Always strop the razor after use.

5. If much section-cutting is being done, it is advisable to have three razors in use. Make use of each in rotation and rest each for two consecutive days.

Refractive Indices of Examination Media.*

		(Order.)
Air	1.000	(1)
Alcohol, ethyl (absolute = 100%)	1.367	(6)
Alcohol, methyl	1.323	(2)
Benzene	1.504	(12)
Canada balsam, solid	1.535	(20)
Canada balsam, in xylene	1.524	(18)
Cedar-wood oil (not thickened)	1.510	(13)
Clove oil	1.533	(19)
Egg albumen soln.	1.350	(5)
Euparal (Gilson)	1.484	(10)
Flatters' and Garnett's Neutral Mountant	1.515	(15)
Glass (crown)	1.518	(16)
Glycerine (100%)	1.470	(9)
Glycerine (50% aq.)	1.397	(7)
Gum Dammar	1.520	(17)
Gurr's Neutral Mountant	1.510	(13)
Hyrax	1.710	(22)
Lacto-phenol	1.440	(8)
Lenzol (Gurr's)	1.510	(13)
"Mersol" (Flatters')	1.511	(14)
Phenol	1.549	(21)
Sirax (dry)	1.810	(23)
Turpentine	1.470	(9)
Water, distilled	1.336	(3)
Water, sea	1.343	(4)
Xylene	1.497	(11)

Note: (a) To enhance visibility, employ media of refractive index lower than that of Canada balsam.

* Reproduced, with modifications, by permission of Messrs. J. and A. Churchill from Bolles-Lee's *Microtometist's Vade-Mecum*.

- (b) To enhance transparency, employ media of refractive index higher than that of Canada balsam.

Renaut's Eosin-Hæmatoxylin.

Nuclei (plant).

Eosin Y (conc. aq.) (p. 244)	30 c.c.
Hæmatoxylin (satd. alc.) (p. 252)	40 c.c.
Potash alum (satd. glycerine)	130 c.c.

Leave unstoppered for several weeks until alcohol has evaporated. Filter.

Ringer's Solution. See "Saline Solutions" (p. 281).

Ringin Media.

(See also "Cement," pp. 234, 235, and "Ringin" in Chapter III, p. 47.)

(i) Bause's Ringin Varnish.*

Does not crack, "run in," or "spread."

- 1° Place a quantity of ordinary flaked shellac in an earthenware or enamel vessel.
- 2° Cover the shellac with methylated spirit.
- 3° Place the vessel in a saucepan (whose rim should be lower than the rim of the inner vessel) and pour water into the saucepan until the levels of the liquids in inner and outer vessels are the same.
- 4° Heat the water and meanwhile keep the shellac and alcohol stirred with a glass rod or soft-wood stick. *Be ready to lift the saucepan off the source of heat as soon as the methylated spirit is on the point of boiling.*
- 5° Keep stirring until all the shellac is dissolved. Ignore any residue of stringy material.
- 6° Warm another earthenware or enamel vessel and filter the shellac solution into it, through several layers of muslin.

* Private communication to the author.

7° Add to the filtered shellac solution, 25% of its volume of Venetian turpentine. Reheat in the water in the saucepan and keep stirring till mixture is complete. On cooling, there will result a thick jelly which may be stored indefinitely in a tightly corked vessel.

8° When required for ringing, thin out a suitable quantity with methylated spirits until it is of the correct consistency for the purpose.

N.B. If a black varnish is required, add to every 4 oz. of methylated spirit used for thinning, one salt-spoonful of "spirit-black" (obtainable from oil and colour merchants).

9° Reheat the thinned varnish and allow to cool. (Reheating after thinning makes the varnish more tenacious.) The varnish is now ready for use.

(ii) *Krönig's Deckglaskitt*.*

(a) Wax 2 parts.

Melt, and add gradually,

Colophonium resin 7-9 parts.

Mix thoroughly, filter, and cool.

(b) *To apply*. Heat an L-shaped metal rod in a flame, dip into the vessel containing the medium and apply to the edge of cover-slip.

(iii) *Gray's Sealing Medium for Fluid Mounts*.†

(a) Melt together

Lanoline (anhydrous) 4 parts.

Resin 8 parts.

Canada balsam (dry) 1 part.

(b) Use.

(α) *For circular cover-slips*.

1° Fit a handle on to a piece of brass-tubing of bore very slightly in excess of the diameter of the cover-slip.

* After Carleton, *Histological Technique*, Oxford Medical Publications.

† Adapted from *The Microscope Record*, No. 33; Sept. 1934. Messrs. W. Watson & Sons, Ltd.

2° Heat the tubing, dip into mixture, and apply over the cover-slip. The cement hardens immediately.

(β) For square cover-slips.

1° Heat a thick steel knitting needle and flatten one end to make a small spatula.

2° Bend to a convenient shape.

3° Apply cement by means of heated tool.

(iv) See also "Gold Size" (p. 250); "Cement" (p. 234); "Zinc White" (p. 290); and "Ringing" (p. 47).

Rosaniline. See "Basic fuchsin" (p. 228).

Ruthenium Red (Ammoniacal ruthenium sesquichloride).

Pectic compounds (mucilages and gums). Does not stain mucilage derived from cellulose. Stains chromatin well; cytoplasm feebly; cellulose not at all.

Ruthenium red 0.01 gr.

Distilled water 100 c.c.

Keep in the dark.

Safranin O. (Cotton Red. Gossypimine.) C.I. No. 841.

Nuclear. (A basic dye.) Do not use in glycerine jelly mounts. Chromatin (→ red); Cutin (→ pink); Elastic fibres; Fresh animal tissue; Fungi; Lignin (→ cherry-red) (counterstain with Delafield's hæmatoxylin, aniline blue W.S., picric-aniline blue W.S., or light green S.F. yellowish in clove oil); Nuclei (plant and animal) (→ red); *Opalina*; Plant tissue (fixed sections) (counterstain with hæmatoxylin or light green S.F. yellowish); Protozoa; Sieve plates (temp.); Spermatozoa; Starch (→ pink); Unlignified tissue (→ brown-red); Xylem (→ cherry-red) (counterstain as for lignin).

(a) Safranin O 1 gr.
Alcohol (50%) 99 c.c.

- (b) See also "Babe's safranin" (p. 228).
 (c) *Formula for use in Breinl's stain:*
 Safranin O, sat. aq. soln. 50 c.c.
 Safranin O (alcohol sol.),
 sat. alc. (100%) soln. 50 c.c.
 Aniline oil few drops.
 Best if allowed to ripen for 3 months.

Saline Solutions.

- (i) *For temporary bathing of tissues for microscopic and other purposes, use saline solutions isotonic with the cell contents.*

- (a) *Ringer's solution. (Isotonic salt solution).*

Best for general use.

Potassium chloride	0.042 gr.
Calcium chloride	0.024 gr.
Sodium bicarbonate	0.02 gr.
Distilled water	100 c.c.

Add:

- (α) *For mammalian tissue, except blood,*

Sodium chloride	0.90 gr.
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- (β) *For frog tissue, except blood,*

Sodium chloride	0.75 gr.
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- (γ) *For invertebrate tissue, and vertebrate blood,*

Sodium chloride	0.60 gr.
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- (b) *Isotonic sodium chloride solutions.**

- (α) *For mammalian tissue except blood,*

Sodium chloride	0.90% aq.
---------------------------	-----------

- (β) *For frog tissue, except blood,*

Sodium chloride	0.75% aq.
---------------------------	-----------

- (γ) *For invertebrate tissue, and vertebrate blood,*

Sodium chloride	0.60% aq.
---------------------------	-----------

* Sometimes loosely called "Normal Salt Solutions." The term normal is best avoided as it may lead to confusion with its use in the chemical sense when solutions are being prepared.

- (ii) To discharge *Hydra nematocysts*.
Sodium chloride 5.0% aq.
- (iii) For fixing and preserving. See
"Formol-Saline" (p. 248).

Scarlet Red. See "Sudan IV" (p. 284).

Scharlach R. See "Sudan IV" (p. 284).

Schaudinn's Fluid.

For fixing protozoa.

Mercuric chloride (sat. aq.)	100 c.c.
Alcohol (100%)	50 c.c.

Schulze's Solution. (Chlor-zinc-iodine.)

Cellulose—a specific stain (temp.) (→ blue-violet); Cutin (temp.) (→ yellow-brown); Hyphæ (cell wall) (temp.); Lignin (temp.) (→ yellow-brown); Starch (temp.) (→ blue); Suberin (temp.) (→ yellow-brown).

Place sections in water for a few minutes before staining.

(a) Zinc chloride	30 gr.
Potassium iodide	5 gr.
Iodine	1 gr.
Distilled water	14 c.c.

(b) *Leach's formula*.*

Hydrochloric acid (conc.) satis.

Zinc, add to the acid until effervescence ceases, and excess zinc remains.

Evaporate liquid until of consistency of conc. sulphuric acid. Now add

Potassium iodide to saturation.

Allow liquid to stand.

Decant supernatant liquid. Add

Iodine to saturation.

Decant supernatant liquid.

Keep in the dark. Make up freshly from time to time.

* After Leach, *Textbook of Practical Botany*, Methuen.

Scott's Tap-Water Substitute. See "Tap-Water Substitute" (p. 285).

Sealing Media. See "Ringing Media" (p. 278).

Silver Nitrate.

- (i) Cell outlines; Cement substance; Chlorine ions; Stomata in pavement epithelium [see "Epithelia" (p. 151)].
0.5% aq. soln.
- (ii) *For Cajal's method for Golgi bodies* (p. 166).
1.5% aq. soln.

Slide Holder.*

Useful when dealing with quantities of slides in the same reagent. Stretch Woolworth's "Chest-expander" springs (which are about 2 cm. in diameter) until there is a permanent gap (slightly less than the thickness of a slide) between each coil. Cut the spring into lengths of convenient size to hold the required number of slides when placed in the containing vessel.

Slides—To Clean. See "Glassware—To Clean" (p. 249).

Sodium Chloride. See "Saline Solutions" (p. 281).

Sodium Metasilicate.

For cleaning the white deposit from glassware. 5% aq. soln.

Sodium Nitrate.

1.5% aq. soln.

Sodium Sulphate.

For washing after decalcification. 5% aq. soln.

* J. McCloy, *School Science Review*, XX, 80, 607; June 1939.

Sodium Thiosulphate (Hypo.).

For removal of iodine after iodine-treatment of tissue fixed with mercuric chloride.

Sodium thiosulphate	7.5 gm.
Alcohol (96%)	100 c.c.
Distilled water	900 c.c.
Thymol	1 crystal.

Solutions, Dilution of. See "Dilution of Liquids, Table for " (p. 240).

Sudan G. See "Sudan III " (below).

Sudan Red. See "Magdala Red " (p. 262).

Sudan III. (Fat ponceau G. ; Sudan G.) C.I. No. 248.

(An acid dye.) Cutin (slow) ; Fat (\rightarrow red). See "Fat " (p. 153).

Sudan III	excess.
Alcohol (70%)	50 c.c.
Acetone	50 c.c.

Filter when soln. is satd. and keep in a well-stoppered bottle.

Sudan IV. (Fat ponceau R. ; Scarlet Red ; Scharlach R.) C.I. No. 258.

Cutin. Fat.

Sudan IV	5 gr.
Alcohol (70%)	95 c.c.

Sulphated Fatty Alcohols. See "Glassware—To Clean " (ii) (b) (p. 249).

"Susa."

A good general fixative for animal tissue. Does not harden unduly.

Mercuric chloride (sat. aq.)	50 c.c.
Acetic acid (glacial)	4 c.c.
Trichloroacetic acid	2 c.c.
Formaldehyde (40%)	20 c.c.
Distilled water	24 c.c.

Fix for 3-4 hours. Wash in alcohol (50%) to which iodine has been added to give a fairly deep brown colour (12 hours). If colour disappears, add more iodine (to remove mercuric chloride). Transfer to alcohol (70%) till required for use.

Syrup.

As an immersion medium for high-power lenses. Dilute golden syrup with an equal volume of distilled water.

Tannic Acid.

To discharge trichocysts of Paramecium. 1.0% aq. soln.

Tap-Water Substitute.* (Scott's.)

For "blueing" sections stained in Delafield's haematoxylin.

Solution A.

Sodium bicarbonate	3.5 gr.
Tap-water	500 c.c.

Solution B.

Magnesium sulphate	20 gr.
Tap-water	500 c.c.

Mix equal volumes of Solutions A and B. Add a crystal of thymol to the stock mixture.

Taxidermy (Preservatives for).

(i) See "Bécœur's Arsenical Soap" (p. 229) and "Browne's Soap" (p. 232).

(ii) Burnt alum 1 lb.

Potassium nitrate 4 oz.

Mix thoroughly. See "Skins of Animals," (i), p. 321.

* After Carleton, *Histological Technique*, Oxford Medical Publications.

Thionin. C.I. No. 920.(a) *For van Wijhe's method for cartilage* (p. 140).

Thionin.	0.5 gr.
Alcohol (70%)	100 c.c.
Hydrochloric acid (conc.)	1 c.c.

(b) See also "Carbol-Thionin" (p. 233).

Toluidin blue O. C.I. No. 925.*For van Wijhe's method for cartilage* (p. 140).

Toluidin blue O	0.25 gr.
Alcohol (70%)	100 c.c.
Hydrochloric acid (conc.)	0.5 c.c.

Toning Solution.*For use in Cajal's method for Golgi bodies* (p. 166).*Solution A.*

Sodium thiosulphate	3 gr.
Ammonium sulphocyanide	3 gr.
Distilled water	100 c.c.

Solution B.

Gold chloride	1 gr.
Distilled water	100 c.c.

Keep separately. Just before use mix equal volumes of each in a test-tube, to give a sufficient quantity to pour over the sections on the slide.

Turpentine-Phenol. See "Carbol-Turpentine" (p. 233).**Venetian Turpentine.**

Venetian turpentine resin is thinned with an equal volume of absolute alcohol, in a flask fitted with a calcium chloride tube on a water-bath. Filter in a desiccator. To 10 c.c. of the thick Venetian turpentine so obtained add, alcohol (100%), 90 c.c.

Note: Venetian turpentine is very apt to take up water from the atmosphere and become cloudy. Preparations are best left in a desiccator containing soda-lime.

Vesuvium. See "Bismarck Brown Y" (p. 230).

Water Blue. See "Aniline Blue W.S." (p. 226).

Water-Soluble Eosin. See "Eosin Y" (p. 244).

Wax. See "Paraffin Wax" (p. 270).

Weigert's Iron Hæmatoxylin.

For general and cytological work. Stain 10-15 mins.
If necessary, differentiate with acid alcohol.

Solution A.

Hæmatoxylin	1 gr.
Alcohol (95%) or (96%)	99 c.c.

Solution B.

Ferric chloride (30% aq.)	4 c.c.
Hydrochloric acid (s.g. 1.124)	1 c.c.
Distilled water	95 c.c.

Allow solution A to ripen for a few days, but use it within 6 months. Just before use, mix equal volumes of solutions A and B.

Weigert's Stain.

Elastin (→ blue-black); Cartilage; Connective tissue.

Basic fuchsin	1 gr.
Phenol	2 gr.
Distilled water	100 c.c.

Dissolve and bring to boiling point in a large bowl.

When boiling, add, in small amounts, to precipitate the dyestuff,

Ferric chloride (30% aq.)	12.5 c.c.
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Boil and stir, for another 10 mins. Cool. Filter.

Wash ppts. on filter till runnings are colourless.

Dry ppt. To make the solution:

Dry precipitate	0.75 gr.
Alcohol (90%)	100 c.c.

Boil under reflux condenser for 20 mins. Cool.

Filter. Add:

Hydrochloric acid (conc.)	2 c.c.
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Wright's Stain.

For blood films. See "Blood" (c), (d), and (e) (p. 135).

Wright's stain (dry)	0.1 gr.
Methyl alcohol (100% ; neutral ; acetone free)	60 c.c.

Xylene.

When used for clearing, shows "milky" if there is the slightest trace of water remaining in the tissue. Replace in fresh alcohol (100%), and use xylene-phenol (p. 289), or, better still, benzene-phenol (p. 229).

Xylol. See "Xylene" (above).

Xylene-Acetone. See "Acetone-Xylene" (p. 221).

Xylene-Alcohol. See "Alcohol-Xylene" (p. 224).

Xylene-Eosin Y (and Xylene-Erythrosin bluish).

For differential staining (see pp. 28, 199).

1° Make 1% aq. solution of eosin Y (p. 244) [or, erythrosin bluish (p. 246)].

2° either, (a) For every 1 gm. of eosin Y in solution add 3.5 c.c. of 10% soln. of hydrochloric acid of s.g. 1.16.

or, (b) For every 1 gm. of erythrosin bluish in solution add 2.5 c.c. of 10% soln. of hydrochloric acid of s.g. 1.16.

3° Allow ppt. (immediate) to settle for 24 hours.

4° Pipette off clear liquid.

5° Add pure xylene to residue (which is the solid, free, dye-acid) at rate of 200 c.c. xylene for each 1 gm. of dyestuff used.

6° Mix by stirring. (Avoid strong shaking with erythrosin bluish, or emulsion may form.)

7° Pour into separating funnel. Agitate gently as long as any solution occurs.

8° Allow to settle. Run off the colourless xylene-solution of the free, dye-acid.

[If stronger solution required, filter original ppt. (3°) on to asbestos in a Gooch crucible; air dry; shake with dry xylene in a mechanical shaker.]

Xylene-Erythrosin bluish. See under "Xylene-Eosin Y" (p. 288).

Xylene-Methylene Blue (and Xylene-Nile Blue Sulphate).

For differential staining (see pp. 28, 199).

1° Make 1% aq. soln. of methylene blue (p. 265) [or, Nile blue sulphate (p. 268)].

2° For every one gram of dyestuff in solution add 2.5 c.c. of sodium hydroxide (10%).

3° Allow ppt. (several hours) to settle 24 hours.

4° Pipette off clear liquid.

5° Proceed with the residue (which is the solid, free, dye-base) as under "Xylene-Eosin Y," 5° (p. 288) (*et seq.*) and thus obtain the xylene-solution of the free, dye-base.

Xylene-Nile Blue Sulphate. See under "Xylene-Methylene Blue" (above).

Xylene-Phenol.

Partly overcomes the trouble of "milky" xylene due to the presence of water.

Xylene	100 c.c.
Phenol	5 gr.

Xylene-Wax.

Xylene	50%
Paraffin wax (M.Pt. 50° C.)	50%

Zenker-Formol.

- | | |
|---------------------------------|----------|
| (i) Mercuric chloride | 5 gr. |
| Potassium dichromate | 2.5 gr. |
| Sodium sulphate | 1 gr. |
| Distilled water | 100 c.c. |
- Just before use, add :
 Formaldehyde (40%) 5 c.c.
- (ii) Fix for 12-14 hours. See "Zenker's Solution."
 (iii) Do not use for plant material.

Zenker's Solution.

Mercuric chloride	5 gr.
Potassium dichromate	2.5 gr.
Sodium sulphate	1 gr.
Acetic acid (glacial)	5 c.c.
Water	100 c.c.

Use as Zenker-formol ; after washing in water, place tissue in alcohol (70%) to which iodine has been added. If colour disappears, add more iodine to remove mercuric chloride.

Ziehl's Carbolic-Fuchsin.

Nuclear. Bacteria and their spores ; fungi.

Basic fuchsin (see p. 228).	1 gr.
Phenol (5% aq.)	100 c.c.
Alcohol (100%)	10 c.c.

Keeps well.

Zinc White.

Zinc Oxide	10 parts.
Turpentine	10 parts.

Rub up the zinc oxide with the turpentine and add :
 Gum Dammar in turpentine 80 parts.
 The solution of gum Dammar should be of the consistency of treacle.

CHAPTER VIII

SOURCES AND CULTURE OF MATERIAL *

Though obviously not strictly a part of microtechnique, it seems essential to have material on which to work, and the writer has sometimes suffered no little inconvenience by not being able to lay his hands on the right material at the right time. As with the rest of the book, these notes have been culled from various sources. They refer mostly to animal tissue. A friendly acquaintance with a nurseryman (particularly if he be a hot-house specialist) usually paves the way to a ready (and often regular) supply of a wide range of botanical material; and animals often arrive in too great quantity in jam-jars at the hands of junior pupils.

If it is not convenient to use all the material at once; it may be necessary to preserve it for future use. Hence the notes on preservation in Chapter IX (p. 312).

Algæ.

Culture in Knop's solution, or in Bristol's solution.

Amœba.

- (i) Dealers. On arrival, allow the tube of culture to stand undisturbed for 1 *hour*. Pipette a small quantity of culture from the *bottom* of the tube and examine under the microscope (low-power objective; light stopped down).

* The writer is indebted to the Editor of the *School Science Review* for permission to reproduce, in a modified form, much of the information in this chapter.

If the culture is alive, it may be propagated. [See "Protozoa" (p. 309).] Feed by placing one or two boiled wheat grains in the Petri dish. Do *not* overfeed, because bacteria and infusoria will become too abundant.*

- (ii) Surface mud and bottom silt from ditches and ponds. Tie one end of a piece of thread to a cork, and the other end to a small rubber band which is placed round two cover-slips. Sink the slips to the bottom of the pond. Examine the water film contained between the slips.
- (iii) Place garden soil in pond water and examine daily.
- (iv) Place a chopped earthworm in water and examine later.
- (v) Place chopped *Elodea canadensis* in water and examine surface scum after several days.
- (vi) See "Daphnia" (iii) (e) (p. 295), but inoculate with *Amœba*.
- (vii) *Hay infusion*.

Hay (chopped)	10 gr.
Distilled water	1 litre.

Steam for $\frac{3}{4}$ hour. Filter. Add a few drops of sodium hydroxide to make filtrate neutral to litmus. Cool. Place a little in a large Petri dish and inoculate with a little soil. Leave for a few days. Once a month add one or two crushed wheat grains.

Anodon.

- (i) Dealers.
- (ii) From the floor of ponds and canals.
- (iii) On arrival from a dealer, keep in running water in a dark place. May be kept in larger aquaria.

Anopheles.

- (i) *Eggs and larvae*, by a bolting-silk tow-net attached to a bottle towed in ponds, water butts, etc.

* Adapted from *Gerrard's Bulletin*, June 1939. T. Gerrard & Co.

- (ii) *Adults* caught at rest by placing over them a specimen tube with a piece of chloroform-soaked cotton wool at the bottom. See "Killing Bottle" (iii) (p. 258).

Ascaris.

- (i) Dealers.
- (ii) Abattoirs—take a vessel of formaldehyde (5%), and also try to keep live ones for a short time in warm saline (0.6%) in a vacuum flask.
- (iii) Lungs of *Rana*.

Astacus.

- (i) Dealers.
- (ii) Catch at night, in nets in streams in limestone districts.
- (iii) On arrival after a journey in damp grass, place the animals in a shallow vessel and half cover with water. This enables them to exclude air from gill covers gradually.
- (iv) May be kept in an aquarium with a stony bottom.

Asterias.

- (i) Dealers.
- (ii) Rock pools.
- (iii) Fishermen.
- (iv) Keeping in sea-water tanks not easy, except by experienced aquarists. Feed on live shell-fish.

Atax ypsilophorous.

In mantle cavity of *Anodon*.

Bacteria.

- (i) All apparatus must be sterilized—for method see text-books, but a simple method is to use a household steaming-saucepan. Place the apparatus in the top compartment for *half an hour on three successive days*. Do not remove the lid in the meantime.

(ii) Culture media : Raisin gelatin (p. 275) ; Dox's Agar (p. 242) ; Potato Agar (p. 274).

*(iii) *To culture in the dark.* Use a plaster-of-Paris box with a plaster lid, either, moistened from the outside from time to time, or, placed in a shallow reservoir of water.

(iv) See also "Hanging-Drop Cultures" (p. 302).

Balantidium.

In bladder of *Rana*.

Bodo.

Culture in an infusion of putrid fish.

Cladocera. See "Daphnia" (p. 295).

Cladophora.*

To obtain swarm spores. Take from rapidly running water and lay in $\frac{1}{2}$ " depth of water in a shallow vessel overnight. Fix with osmium tetroxide (1%).

Clepsine. See under "Hirudo" (p. 304).

Columba.

- (i) Dealers.
- (ii) Fishmongers.
- (iii) Live specimens : kill with chloroform vapour.

Copromonas.

Large intestine of *Rana*.

Culex. See "Anopheles" (p. 292).

Cyclops. See "Daphnia" (p. 295).

* After Strasburger, *Handbook of Practical Botany* (English edn.), Allen and Unwin.

Daphnia.

- (i) Dealers.
- (ii) Dredge from semi-stagnant ponds in a very fine mesh net.
- (iii) Culture in shallow water. Feed on one of following media :
 - (a) Dead elm leaves.
 - (b) Egg yolk in water. Shake up, leave in a warm place *for some days*. Add a few drops of the pabulum *twice per week*.
 - (c) Fowl dung 3 gr.
 Water 1,000 c.c.
 Boil. Filter. Expose to sunlight. Add 1 part filtrate to 9 parts filtered pond water. The *Daphnia* are kept in this liquid.
 - (d) Add Horlick's Malted Milk powder to water.
 - (e) Boil 20 wheat grains in 1 litre of water. Cool. Inoculate with *Daphnia*.* Add fresh boiled grains *once a month* and sub-culture *once in three months*.

Dealers.† (Material supplied is shown in brackets.)

Aquaria Supplies Co., 106A, Camberwell Road, London, S.E.5.
 (Aquaria and fish.)

Backhouse & Son, Nurserymen, York. (Bot. material.)

Baird & Tatlock, 14, Cross St., Hatton Garden, London, E.C.1.
 (Apparatus.)

Baker, C., 244, High Holborn, London, W.C.1. (Microscopes, etc., and micro. preps.)

Batt, W. J., Elmhurst, Hullbridge Rd., South Woodham, Chelmsford, Essex. (Biological models.)

Bausch & Lomb Optical Co., Ltd., Africa House, Kingsway, London, W.C.2. (Microscopes.)

* If this method is used for *Amæba*, inoculate, either, with a pure culture of *Amæba*, or, by adding a teaspoonful of garden soil.

† This list has been compiled from various sources, including especially Stork and Renouf's *Fundamentals of Biology*, Murray; and Mangham and Sheriff's *A Second Biology*, Sidgwick and Jackson; to which the author owes grateful acknowledgment.

- Bause, C. F., 11, Fellbrook Avenue, Acomb, York. (*Micro. preps. ; mounting materials ; specimens.*)
- Beck, R. & J., Ltd., 69, Mortimer St., London, W.1. (*Microscopes.*)
- Becker, F. E. & Co., Hatton Wall, London, E.C.1. (*Apparatus.*)
- Biddolph, A., "Green Belt," London Rd., Merstham, Surrey. (*Zoo. material ; preps. ; micro. preps.*)
- Bolton, T., Newhall St., Birmingham. (*Fresh-water algae.*)
- Botanical Supply Agency, Rhydyfelin, Aberystwyth. (*Bot. material, fresh and preserved ; micro. preps.*)
- Brady & Martin, Northumberland Rd., Newcastle-on-Tyne. (*Apparatus.*)
- British Drug Houses Ltd., Graham St., London, N.1. (*Chemicals ; stains.*)
- British Mosquito Control Institute, Hayling Island, Hants. (*Mosquitoes ; diagrams ; mosquito control apparatus.*)
- Broadhurst, Clarkson, & Co., 63, Farringdon Rd., London, E.C.1. (*Second-hand microscopes ; micro. preps.*)
- Bunce, Forest Green, Maidenhead, Berks. (*Certain micro. preps.*)
- Child, B. T., 113, Pentonville Rd., London, N.1. (*Aquaria and accessories.*)
- City Sale and Exchange (1929) Ltd., 54, Lime St., London, E.C.3. (*Second-hand microscopes.*)
- Clarksons, 338, High Holborn, London, W.C.1. (*Second-hand microscopes.*)
- Cork University Biological Station, Lough Ine, Co. Cork, Eire. (*Marine, fresh-water, and terrestrial plants and animals.*)
- Cura & Sons, Bath Court, Warner St., Rosebery Av., London, E.C.1. (*Aquaria supplies.*)
- Darlaston, H. W. H., Freer Road, Birchfields, Birmingham. (*Micro. preps. ; mounting customer's material.*)
- Flatters & Garnett, Ltd., 309, Oxford Rd., Manchester, 13. (*Micro. preps. ; certain living material ; collecting and other apparatus.*)

- Freshwater Biological Association of the British Empire,
Wray Castle, Ambleside, Westmorland. (*Dead and living
fresh-water animals and plants.*)
- Gallenkamp & Co., Ltd., 17-29, Sun St., Finsbury Sq.,
London, E.C.2. (*Living and dead plants ; apparatus.*)
- Galloway, J. C., and Jones, J. Morgan, Botanical Supply
Agency, Rhydyfelin, Aberystwyth. (*Fresh and preserved
bot. material ; micro. preps.*)
- George, W. & J. Ltd., 157, Great Charles St., Birmingham, 3.
(*Apparatus ; micro. preps.*)
- Gerrard, T. & Co., 46A and 48, Pentonville Rd., London, N.1.
(*Dead and living bot. and zoo. material ; preparations ;
models ; micro. preps. ; apparatus ; chemicals.*)
- Griffin & Tatlock, Ltd., Kemble St., Kingsway, London, W.C.2.
(*Micro. preps. ; apparatus.*)
- Gurr, G. T., 136, New King's Rd., Fulham, London, S.W.6.
(*Biological reagents ; stains ; instruments.*)
- Haig, L., Beam Brook, Newdigate, Surrey. (*Aquarium
material.*)
- Harris, Philip, & Co., Ltd., 144-146, Edmund St., Birming-
ham, 3. (*Apparatus ; micro. preps.*)
- Hawksley & Sons, Ltd., 17, New Cavendish St., London, W.1.
(*Microscopes and accessories.*)
- Hick, P., Athol House, Scarborough. (*Exotic butterflies and
moths.*)
- Janson & Sons, 44, Great Russell St., London, W.C.1. (*Ento-
mological specimens.*)
- Jessop, H., 30, Beckmead Av., Kenton, Middlesex. (*Amæba.*)
- Land, S., 5, Progress St., Castleton, nr. Rochdale, Lancs.
(*Live and dead bot. and zoo. material.*)
- Leitz, E. (London), 20, Mortimer St., London, W.1. (*Micro-
scopes and accessories.*)
- Leng, 183, Manchester Drive, Leigh-on-Sea. (*Micro. preps.*)
- Marine Biological Station, Citadel Hill, Plymouth. (*Live and
dead marine material.*)
- Midland Fisheries, Nailsworth, Glos. (*Fresh-water material.*)
- Molloy, J. E., Dell Cottages, Cowley, Middlesex. (*Certain
aquarium material ; frogs ; skeletons.*)

- Morley, Miss M. R., Durnfords, Chiddingfold, Godalming, Surrey. (*Simple bot. specimens.*)
- Murby, Thos., & Co., 1, Fleet Lane, Ludgate Circus, London, E.C.4. (*Geological and palaeontological specimens and apparatus.*)
- Newman, L. W., Bexley, Kent. (*British Lepidoptera.*)
- Newton & Co., 72, Wigmore St., London, W.1. (*Microscopes.*)
- Palmer, C. F. (London), Ltd., 63, Effra Rd., Brixton, London, S.W.2. (*Recording drums and other physiological apparatus.*)
- Port Erin Biological Station, Isle of Man. (*Marine material.*)
- Purves, E., 107, The Vale, Golders Green, London, N.W.11. (*Animal material; injections; skeletons.*)
- Reynolds & Branson, Leeds. (*Apparatus.*)
- Richards, G. H., 48, Sydney St., Chelsea, London, S.W.3. (*Minerals; shells; geological specimens.*)
- Rose, Herbert G., 9, Six Bells Lane, Sevenoaks, Kent. (*Aquarium plants; living pupae; mantis; stick insects; rare arachnida.*)
- Scottish Marine Biological Station, Keppel Pier, Millport, Cumbrae. (*Marine material.*)
- Smith, G. Pugh, Dept. of Education, University College, Cardiff. (*Drosophila melanogaster.*)
- Surrey Trout Farm, Haslemere. (*Fresh-water material.*)
- Swift, J., & Son, Ltd., 81, Tottenham Court Rd., London, W.1. (*Microscopes.*)
- Trotman, P. A., University Museum, Oxford. (*Red-eyed gammarus.*)
- de Von & Co., King's Cross Rd., London, N. (*Livestock, including frogs, guinea pigs, rabbits.*)
- Wadsworth, A., Selbourne, High Lane, nr. Stockport, Cheshire. (*Living material.*)
- Ward, F., Histon, Cambs. (*Taxidermy.*)
- Watkins & Doncaster, Strand, London, W.C. (*Live and dead insects; collecting apparatus.*)
- Watson & Sons, 313, High Holborn, London, W.C.1. (*Microscopes and accessories; instruments; micro. preps.*)
- Williams, E., 4, Britannia Row, Ilfracombe, N. Devon. (*Dogfish—in lots of not less than 1 dozen.*)

Williams, R. W., and van Houtte, Jan, Ltd., Old Sorting House, Lampmead Rd., Lee, London, S.E.12. (*Plants and bulbs.*)

Drosophila.

(i) Dealers.

(ii) Culture media. (Suggested by Dr. Pugh Smith.)

(a) Maize meal 400 c.c.

Raw sugar (= unrefined sugar
molasses) 100 c.c.

Treacle 100 c.c.

Thoroughly mix the above. Next have ready,

Water (boiling) 500 c.c.
and

Agar-agar (powder) 10 gr.

Add about 30 c.c. of boiling water to the agar, and boil. Proceed to add a further 30 c.c. of boiling water, and boil the whole again, and so on till all the water is added.

Next add the meal-sugar-treacle mixture to the agar solution. Stir thoroughly. Heat until the mixture begins to stiffen (about 1 *minute*). Add,

Cold water 100 c.c.

Stir thoroughly. Pour into *sterilized* bottles or specimen tubes to about $1\frac{1}{2}$ " depth. Add, to each vessel,

Yeast suspension about 0.25 c.c.

To allow gases to escape, remove a little of the medium from the side of the bottle. Place a piece of absorbent tissue paper (toilet paper) in the vessel. Plug with cotton wool and re-sterilize as in "Bacteria" (i) (p. 293).

(b) Agar-agar (powder) . . 1.5 teaspoonsful.

Bananas (skinned) . . . 3

Mix the agar and bananas thoroughly. Add,

Water 1.5 pints.

Boil and stir thoroughly. As in (a), above, place in vessel, add yeast suspension, toilet paper, and plug.

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- (c) Agar-agar (powdered) 30 gr.
 Water 1,000 c.c.

Boil the water and agar and then add cornflower, until the mixture is of the consistency of porridge.

Then add

Black treacle 1 teaspoonful.

N.B. To prevent growth of moulds on above culture media, add

Nipagin M,* to every 1,000 c.c. of medium, 1.5 gr.

(iii) Culture the flies at 25° C.

(iv) For suggested experiments see G. Pugh Smith, *School Science Review*, XVIII, 70, 260, December 1936; and P. G. Fothergill, *Biology*, V, 1, 14, Summer Term, 1939.

Echinorhyncus.

Encysted forms as egg-shaped orange bodies of gut of *Astacus*.

Enchytræ † (White Worm; Pot Worm).

1° Fill a wooden box (8" × 6" × 3" deep), provided with drainage holes (covered to prevent loss of soil), with

Fine, well-aerated soil 3 parts.

Leaf mould or peat dust 1 part.

2° Sprinkle oatmeal over the soil; damp slightly; mix the whole.

3° Either, soak bread in water and, when soft, squeeze the water out; or, boil potatoes (without salt).

4° Sprinkle the bread (or potatoes) over the soil, and mix again.

5° Introduce the *Enchytræ*; cover the box with a sheet of glass (raised on blocks about ½" off the box), and (in summer) cover with a sheet of muslin (to exclude flies).

* Obtainable from T. Gerrard & Co., address, p. 297.

† *Enchytræ* and all apparatus for their culture may be obtained from T. Gerrard & Co., by whom the above information was kindly supplied.

- 6° Keep near the ground at room temperature. Do not overfeed, overwater, or overheat. Aerate well, and keep soil alkaline.
- 7° For use for fish feeding, place some *Enchytrae* in water in a Petri dish. Any adherent soil settles, and the worms can be pulled out after bunching.

Euglena.

- (i) Dealers. On arrival, allow the tube of culture to stand undisturbed for 1 hour. Pipette a small quantity of culture from the *surface* of the liquid, and examine under the microscope (low-power objective; light stopped down).

If the culture is alive, it may be propagated. [See "Protozoa" (p. 309).] Feed by placing one or two boiled rice grains in the Petri dish. Do not overfeed. Keep in a light place.

For further culture: add about 60 boiled rice grains to a glass jar (6" × 4" × 2") three parts full of distilled water. When a scum has formed on the surface, add the culture of *Euglena*. Keep in a light place.*

- (ii) Examine green-topped stagnant ponds and ditches, especially those connected with heaps of farm-yard manure.
- (iii) Culture media.

(a) Peptone	0.5	gr.
Glucose	0.5	gr.
Citric acid	0.2	gr.
Magnesium sulphate	0.02	gr.
Potassium phosphate	0.05	gr.
Water	100	c.c.
(b) Ammonium sulphate	0.2	gr.
Potassium phosphate	0.2	gr.
Magnesium sulphate	0.1	gr.
Ferrous sulphate	Trace	
Tap-water	200	c.c.

* Adapted from *Gerrard's Bulletin*, June 1939. T. Gerrard & Co.

Eurotium.

- (i) Place a piece of dry, stale bread under a bell-jar.
- (ii) Prune agar (p. 274).

Fasciola.

- (i) Dealers.
- (ii) Abattoirs.

Fern Prothalli. See "Prothalli of Fern" (p. 309).

Fungi.

- (i) Useful types for culture :
 - (a) *Eurotium* (above); *Mucor* (p. 306); *Penicillium* (p. 306); *Phytophthora* (p. 307); *Pythium* (p. 309); Yeast (p. 311).
 - (b) Saprophytes generally, and some plant parasites—use Potato Agar (p. 274).
 - (c) Coprophilous species. Use Dung Agar (p. 242).
- (ii) Culture media.
 - (a) Brown's Medium (p. 231); Dung Agar (p. 242); Prune Agar (p. 274).
 - (b) See also Dox's Agar (p. 242), Potato Agar (p. 274), and Pasteur's Solution (p. 270).
- (iii) See also "Hanging-Drop Cultures" (below).

Hæmopsis. (Horse leech.) See under "Hirudo" (p. 304).

Hanging-Drop Cultures.*

Useful for the growth of bacteria, fungi, yeast, pollen tubes, etc.

- (i) 1° (a) Cut a frame, about $\frac{1}{8}$ " thick, as advised under "Technique of Mounting" Note (i) (p. 41), and Fig. 2 (p. 42).
- or (b) Cut a hole of suitable size in a piece of cardboard, $\frac{1}{8}$ " thick, and of the same dimensions as the slide.

* Adapted, by permission of the publishers, Messrs. George Allen and Unwin, Ltd., from Strasburger's *Handbook of Practical Botany* (English edn.).

- or (c) If the culture is to be retained for any length of time, use a glass ring, $\frac{1}{8}$ " long. Such a ring may be bought from scientific instrument makers or may be cut from a piece of glass tubing of diameter rather less than that of the circular cover-slip it is proposed to use. The rough ends of the ring must be ground flat and parallel with each other, on a stone, to give a gas-tight fit later.

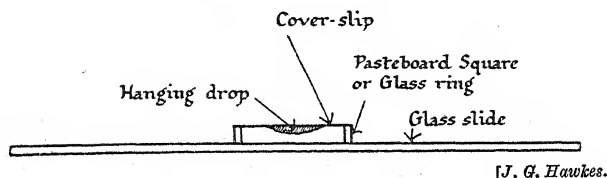


FIG. 21.—Side elevation of arrangement of hanging-drop culture.

- 2° Sterilize a glass slide, a cover-slip, and the glass ring (if used) by dry heat, and sterilize the cardboard by a few minutes' immersion in boiling water.
- 3° (a) Press the wet cardboard on to the sterilized slide in the usual position for mounting.
or (b) Use Canada balsam to cement the glass ring on to the sterilized slide. Smear the upper surface of the ring, very thinly, with vaseline. See also (ii) (b), p. 304.
- 4° Place a drop of the culture medium (with organism therein) on the sterilized cover-slip. If necessary, smear the culture evenly with a sterilized platinum wire.
- 5° Hold the prepared cover-slip by its edge in a pair of sterilized forceps and quickly invert it on the glass ring, or over the hole in the cardboard, and press gently to make gas-tight. See Fig. 21.

- 6° Place the whole in a large Petri dish, and cover to keep it free from dust.
- (ii) *To keep the preparation moist.*
- (a) If cardboard has been used, periodically run a few drops of water on to the slide to keep the cardboard—and hence the preparation—moist.
- or (b) If a glass ring is to be used, place a drop of water on the slide, within the ring, after the latter has been cemented to the slide.
- or (c) * Fashion a box and detachable lid, in plaster-of-Paris, of size convenient for easy manipulation. Place the whole within the box and moisten the latter from time to time.
- (iii) *To observe the effect of gases on a culture.*
- Connect, respectively to a gas-producer and a gas-exhauster, two glass tubes sealed into holes drilled or blown opposite each other in a glass ring fitted to a slide as described in (i) 1° (c), p. 303. Ready-made apparatus to serve this purpose may be bought.

Helix.

- (i) Dealers.
- (ii) *H. aspersa* in damp places in gardens. Hibernates in groups at the bases of dry garden walls under shelter.
- H. pomatia* collected locally, particularly in the Cotswolds.
- (iii) Supply with fresh cabbage leaves and moisture.

Hirudo. (Medicinal leech.)

- (i) Dealers—purchase alive.
- (ii) Pharmaceutical chemists.
- (iii) Ponds and streams. Less common than *Clepsine* and *Haemopsis*.
- (iv) Feed at intervals of 2–3 weeks with raw flesh.

* Adapted, by permission of the publishers, Messrs. George Allen and Unwin, Ltd., from Strasburger's *Handbook of Practical Botany* (English edn.).

Hydra.

- (i) Dealers.
- (ii) Stems of pond weeds, esp. duckweeds and *Elodea canadensis*.
- (iii) Feed on *Daphnia*.

Kerona.

Search tentacles of *Hydra*.

Lepus.

- (i) Dealers. Purchase alive. First anæsthetize in a mixture of chloroform vapour and air. Then kill by increasing the percentage of chloroform.
- (ii) Dead animals may be obtained much more cheaply from farmers, but usually some damage has been done by shot or snare.

Lernæopoda globosa.

In nasal fossæ of some dogfish.

Lernæopoda scyllicola.

In extra-cloacal region of *male* dogfish only.

Liverworts.

- (i) Banks above streams.
- (ii) Damp walls in greenhouses.

Lumbricus.

- (i) Dealers.
- (ii) In dry weather well water the soil and place over it a thoroughly wet sack. Examine towards nightfall and early morning.

Monocystis.

Seminal vesicles and attached to funnels of vasa efferentia of freshly gathered earthworms.

Mucor.

- (i) Scatter some dust on damp bread, under a bell-jar, at room temperature. Leave for a week.
- (ii) Brown's medium (p. 231), or Dox's agar (p. 242).

Nematode.

- (i) See "Ascaris" (p. 293) and "Rhabditis" (p. 310).
- (ii) *Proleptus* sp. found in intestine of *Scyllium*.

Obelia.

- (i) Dealers.
- (ii) Hydroids attached to seaweed in rock pools. Medusoids by tow-net.

Paramecium.

- (i) Dealers. On arrival, allow the tube of culture to stand for 1 hour, undisturbed. Pipette off a small quantity of culture from the *surface* of the liquid, and examine under the microscope (low-power objective; light stopped down).

If the culture is alive, it may be propagated. [See "Protozoa" (p. 309).] Feed by placing three or four boiled wheat grains in the Petri dish. Do not overfeed.

For further culture transfer the thickened, Petri dish culture to a glass jar (6" × 4" × 2") three parts filled with hay infusion [see (iii), below].*

- (ii) Bolting-silk tow-net dragged in ponds and ditches. Examine silt.
- (iii) *Hay infusion*. Boil some chopped hay in water. Add to the liquid a little dry, chopped hay. Bacteria appear first, then *Colpidium*, then *Paramecium*. Avoid breaking surface scum, and subculture twice every three months.

Penicillium.

Dox's agar (p. 242).

* Adapted from *Gerrard's Bulletin*, June 1939. T. Gerrard & Co.

Periplaneta.

- (i) Dealers.
- (ii) Bakehouses by a suitable trap, with hinged top.
- (iii) Supply with water, bread, butter or other fat, bacon rind, and shelter from light.

Phytophthora.

Potato agar (p. 274).

Pinnotheres.

In mantle cavity of *Mytilus*, and in Ascidians.

Pollen Tubes—To Grow.(i) *Method.*

As the growing pollen-tube is negatively aerotropic it is best to use the hanging-drop method (p. 302), though it is not impossible to culture in covered watch-glasses. The temperature should average 21° C. The time taken to grow varies, according to the species, from a *few minutes* to *several hours*. The pollen grains should obviously be fresh. The plants whose pollen-grains are most easily induced to grow tubes are shown in (ii) (b) below, in heavier type.

(ii) *Culture Media.*

Consist, in the main, of solutions of from 5% to 10% *cane* sugar in water, though, according to Strasburger (*Handbook of Practical Botany*), those listed at (a) (p. 308) grow best in water only.

In practice, the percentage of sugar varies with the species. In the same book, from which many of the examples and percentages given in (b) are extracted, Strasburger recommends that spring water * be used and that the culture medium should include 1.5% of gelatin.

* Presumably tap-water?

* (a) *In water only.*

Agapanthus; *Aquilegia* (Columbine); *Lamium*
Galeobdolon (Archangel, Yellow dead-nettle);
Lilium (Lily); *Lobelia*; *Lysimachia* Num-
mularia (Creeping Jenny, Moneywort);
Nicotiana (Tobacco).

* (b) *In cane-sugar solutions.*

For <i>Tulipa</i> (Tulip)	1-3%
„ <i>Allium</i> (Onion)	3%
„ <i>Leucojum aestivum</i> (Snow- flake)	3-5%
„ <i>Narcissus poeticus</i> (Phea- sant Eye)	3-7%
„ <i>Paeonia corallina</i> (Pæony); <i>Papaver</i> (Poppy); <i>Staphy- lea</i> ; <i>Tradescantia</i>	5%
„ <i>Orchidaceæ</i>	5-10%
„ <i>Convallaria majalis</i> (Lily of the Valley)	5-20%
„ <i>Epilobium</i> sp. (Willow-herb); <i>Lychnis Githago</i> (Corn cockle); <i>Geranium</i> sp.; <i>Malva</i> sp. (Mallow); <i>Scilla nutans</i> (Wild Hyacinth)	7-10%
„ <i>Montbretia</i> ; <i>Tritonia</i>	7-5%
„ <i>Gloxinia</i> ; <i>Torenia Asiatica</i>	10%
„ <i>Lathyrus</i> sp. (Sweet- Pea)	10-15%
„ <i>Echeveria retusa</i>	15%
„ <i>Sedum</i> sp. (Stonecrop); <i>Viola tricolor</i> (Pansy)	30%
„ <i>Compositæ</i> ; <i>Iris sibirica</i>	30-40%

* The author is indebted to Messrs. George Allen and Unwin, Ltd., publishers of Strasburger's *Handbook of Practical Botany*, for permission to reproduce the examples given in (a) and many of those in (b).

Polystomella. From Marine Biological Station, Citadel Hill, Plymouth. (Address, p. 297.)

Polystomum.

In bladder of *Rana*.

Proleptus.

In intestine of *Scyllium*.

Prothalli of Fern.

On damp walls, or on soil of flower-pots in fern-houses.

Protococcus.

(i) Dealers.

(ii) Bark of trees.

Protophyta.

Culture medium as *Daphnia* (iii) (c) (p. 295).

Protozoa.*

Culture in sterilized Petri dishes filled to a depth of $\frac{1}{2}$ " with culture (make up loss with distilled water daily), and keep almost covered with the upper half of the dish.

Examine from time to time; cut down food supply if unwanted forms appear; sub-culture when necessary by stirring the culture, transferring half to distilled water in a Petri dish to a depth of $\frac{1}{2}$ ".

See "Amœba" (p. 291), "Euglena" (p. 301), "Paramecium" (p. 306).

Pythium.

(i) Sow cress (*Lepidium sativum*) seeds very closely and keep in a very damp atmosphere.

(ii) Brown's medium (p. 231).

Raia. See "Scyllium" (p. 310).

* Adapted from *Gerrard's Bulletin*, June 1939. T. Gerrard & Co.

- (i) Dealers.
- (ii) Collection. Brickfields, marshy places. In winter look in long grass, under stones, and in mud at pond bottom. Feed on small worms and slugs.

Rhabditis.*

- 1° Kill 3 or 4 earthworms with hot (not boiling) water.
- 2° Remove immediately, and place in a tin box lined with blotting paper and half full of damp earth.
- 3° Keep at summer temperature (approx. 60° F.).
- 4° The first batch of worms is sexually mature at the end of 4-5 days, but, to see cell division after fertilization, females should not be collected till after 6 days at least. Collect with a camel-hair brush.

Rotifers.

Collect in a bolting-silk tow-net from ponds. Rather local in habit.

Scyllium.

- (i) Dealers.
- (ii) Occasionally from fishmongers, but usually arrive eviscerated.

Spirogyra.

- (i) Dealers.
- (ii) Collect from ponds.
- (iii) *To induce conjugation.*†
Between mid-February and May, place in a 2-4% cane-sugar solution in a sunny position.

Stentor.

- (i) Dealers.
- (ii) Collect and culture as *Euglena* (p. 301).

* See Johnson, G. E., *Quart. J. Microscop. Sci.*, 58; 605.

† Strasburger, *Handbook of Practical Botany*, Allen and Unwin.

Tænia.

- (i) Dealers.
- (ii) Dog fæces.
- (iii) Rabbit intestine.
- (iv) Cysticercus from rabbit viscera.

Thread Worms.

In nephridia of *Lumbricus*. See "Rhabditis" (p. 310).

Trichodina.

Search tentacles of *Hydra*.

Volvox.

- (i) Dealers.
- (ii) Collect and culture as *Euglena* (p. 301), and in clear ponds. Rather local in habit.
- (iii) Said to occur in temporary flood-ponds on grassland.

Vorticella.

- (i) Dealers.
- (ii) Examine water weeds and debris.
- (iii) Culture as *Euglena* (p. 301), and *Daphnia* (iii) (c) (p. 295).
- (iv) Sometimes found attached to *Cyclops*.

White Worm. See "Enchytræ" (p. 300).

Yeast.

- (i) Brewery or bakery.
- (ii) Hanging-drop cultures (p. 302) in glucose.
- (iii) *Stir 1 c.c. brewer's yeast into 200 c.c. freshly boiled; cooled (by blowing fresh cold air through it) Pasteur's solution (p. 270). Keep at 20° C. for 12 hours. Sub-culture after 12 hours.

* After Stork and Renouf, *Fundamentals of Biology*, Murray.

CHAPTER IX

PRESERVATION OF MATERIAL *

- Note :* (i) Preferably use stoppered vessels. If screw-top vessels are used, vaseline the screw tops. If corks are used, wax them.
- (ii) The names of the specimen and the preservative may be written in pencil on a piece of white paper, and the paper placed with the specimen in the jar of preservative.

Algæ.

- (i) Bouin's fluid ; formo-acetic-alcohol.
- (ii)† Potassium chrome alum 10 gr.
 Formaldehyde (40%) 5 c.c.
 Water 500 c.c.
- (iii) *Preserving green colour.*
- (a) Camphor water 130 c.c.
 Distilled water 130 c.c.
 Acetic acid (glacial) 20 drops.
 Mix, and add :
 Copper acetate 1 gr.
 Dissolve, and add :
 Glycerine 260 c.c.
 Filter.
- (b) Lacto-phenol 100 c.c.
 Copper acetate 0.2 gr.

* The writer is indebted to the Editor of *School Science Review* and to Messrs. T. Gerrard & Co. (publishers of *Gerrard's Bulletin*) for permission to reproduce, in a modified form, some of the information given in this chapter.

† Adapted from *Gerrard's Bulletin*, May 1939. T. Gerrard & Co.

Amphioxus.

Alcohol (70%).

Anemones (Sea).

Alcohol (70%).

Animal Tissue.(i) *General.*

1° Formol-saline (strong) (p. 248). (10-14 days).

(This also fixes the tissue.)

2° Either (α) Formol-saline (weak) (p. 248).*Permanently.*Or (β) a° Alcohol (30%). (1 day).

b° Alcohol (50%). (1 day).

c° Alcohol (70%) (to which a little glycerine has been added).

Permanently.(ii) *Class specimens. To preserve from lesson to lesson.*

Calcium iodate 2 gr.

Distilled water 2,000 c.c.

Warm till dissolved. The above will keep frogs for 6 months; dogfish for 10 days. It is not suitable for arthropods.

(iii) *Museum specimens, especially insects.*

Pampel's fluid (see p. 270).

This is recommended by Imms* as an excellent fluid for the preservation of biological specimens, especially insects. It is cheaper than ethyl alcohol, does not harden so much, keeps the tissues in better condition and is non-inflammable. Because of the presence of acetic acid it should clearly not be used for animals having any form of calcareous skeleton.

(iv) *Bulk tissue and pathological specimens (gelatin method).**(Permanent and museum mounts.†)*

1° Use fresh tissue, and remove any adherent debris by gentle washing in water. Remove contents of any cavities (intestine, etc.).

* A. D. Imms, *Nature*, 144, 3648, 600. Sept. 30, 1939.

† Adapted from information supplied by the Pathological Department, Cheltenham General Hospital.

PRESERVATION OF ANIMAL TISSUE

- 2° If possible fix (as below) not later than 10-15 minutes after killing. If delay is unavoidable, wrap the tissue in a dry cloth to absorb moisture.
- 3° Cut the tissue so that no piece is larger than a 4" cube.

- 4° Fix in the following solution :

Potassium acetate	30 gr.
Potassium nitrate	15 gr.
Formaldehyde (40%)	200 c.c.
Water	1,000 c.c.

See that all cavities are filled, and, if necessary, ligature.

(12 hours usually ; brain, blood clots, very large specimens 24-48 hours.)

- 5° Immerse in water.

- 6° Immerse in methylated spirit. (To restore colour lost in 4°.)

(Few mins.)

(1-5 hours. Watch each specimen and remove when bright red tint appears. Prolonged treatment spoils.)

- 7° If the specimen does not now look very fresh (e.g. kidney, liver, etc.) cut away a thin slice of tissue to expose a fresh surface.

- 8° Wash off excess alcohol in water.

- 9° Place in Kaiserling's solution (p. 258).

(Days, months, or as a permanent mount.)

(Although this fluid may be used as a permanent mountant, it tends to macerate, and the process should be carried through to completion) :

- 10° Place in permanent storage jars and add melted Kaiserling-gelatin (p. 257).

- 11° Seal storage jars with pitch. [See "Cement" (v) (p. 235).]

(v) See also "Frog-spawn" (p. 316).

Anodon.

Formaldehyde (10%), after inserting wood plug between valves of shell.

Aphrodite.

Formalin (10%).

Aquatic and Marine Animals (Small).

When dead, quickly remove to strong formol-saline.

Arenicola.

Formaldehyde (10%).

Ascaris.

Formaldehyde (2%), or alcohol (70%).

Astacus.

Alcohol (70%).

Asterias.

Formaldehyde (5%). First open dorsal surface of each arm.

Barnacles.

Formaldehyde (10%).

Brain.

- (i) Müller's fluid (p. 267).
- (ii) Formaldehyde (5%).
- (iii) Chrome alum 2.5 gr.
Copper acetate 5.0 gr.
Acetic acid (glacial) 5.0 c.c.
Formaldehyde (4%) 10.0 c.c.
Water 77.5 c.c.

Boil the chrome alum in the water and allow it to dissolve. Remove from the flame and add the copper acetate (powder) and the acetic acid. Stir well. Cool. Add the formaldehyde. The tissue can be regarded as preserved in 14 days.

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Bryozoa. See under "Polyzoa" (p. 320).

Coelenterates.

Quickly transfer to strong formol-saline. See also
"Hydroids" (p. 317).

Crabs.

Formaldehyde (10%).

Crayfish. See "Astacus" (p. 315).

Distomum. See "Fasciola" (below).

Doris.

Alcohol (70%).

Earthworm. See "Lumbricus" (p. 318).

Echinus.

Formaldehyde (8%), after puncturing the test.

Eye (Vertebrate).

Alcohol (70%).

Fasciola.

Formaldehyde (5%).

Fish.

(i) Formaldehyde (10%).

(ii) See also "Scyllium" (p. 320).

Frog Spawn.

(i) Perényi's fluid.*

(ii) Formaldehyde (4%).

(iii) See also "Gatenby's Fluid" (p. 248).

* After Stork and Renouf, *Fundamentals of Biology*, Murray.

Fungi.

Store in Calberla's fluid (p. 232) after treatment by processes 1°-9° of "Fungi" (iv) (b) (p. 157).

Gelatin Method for Animal Tissue.

See "Animal Tissue" (iv) (p. 313).

Gelatinous Tissues, e.g. Snails.

Formaldehyde (4%).

Helix.

- (i) Inject formaldehyde (20%) through the dorsal surface of the foot of a distended snail.
- (ii) Formaldehyde (4%).
- (iii) Inject with, and preserve in,*

Alcohol (100%)	60 c.c.
Formaldehyde (40%)	0.5 c.c.
Water	39.5 c.c.

Hirudo.

Alcohol (60%).

Hydra.

Alcohol (70%).

Hydroids.

Formaldehyde (4%).

Insects.**(i) Small.**

(a) Ethyl acetate (especially useful if the animals are required for dissection because the muscles are kept relaxed).

(b) Alcohol (70%).

(ii) Large.

Dry.

* Adapted from *Gerrard's Bulletin*, May 1939. T. Gerrard & Co.

Jellyfish.

Formaldehyde (10%).

Leech. See "Hirudo" (p. 317).

Lepus.

Formaldehyde (3%).

Limpet. See "Patella" (below).

Lobworm. See "Arenicola" (p. 315).

Lugworm. See "Arenicola" (p. 315).

Lumbricus.

Alcohol (70%).

Marine Organisms (Small). See "Aquatic and Marine Animals, Small" (p. 315).

Mussel. See "Anodon" (p. 315).

Mytilus. See under "Anodon" (p. 315).

Patella.

Formaldehyde (10%).

Pathological Animal Tissue. See "Animal Tissue" (iv) (p. 313).

Pennatula.

Formaldehyde (5%).

Plankton.

Formaldehyde (3%).

Plant Tissue (other than algæ and fungi).

(i) Cut the tissue into pieces 1 cm. \times 1 cm. \times $\frac{1}{2}$ cm.

(ii) *Unfixed material.*

Mixture of equal parts of alcohol (95%), glycerine, and distilled water.

(iii) *Material fixed in chrom-acetic.*

1° Wash in running water.

2° Dehydrate (p. 34) up to, and preserve in, alcohol (70%) (to which a little glycerine has been added).

Note: (a) the addition of glycerine to the alcohol used in storage prevents undue hardening of the tissues.

(b) Material which has turned brown in alcohol may be decolourized by placing it in:

Alcohol (70%)	. . .	100 c.c.
Sulphuric acid (conc.)	. . .	1 c.c.
Potassium chlorate	. . .	few crystals.

for a few days.

Then transfer to, and preserve in:

Alcohol (70%)	. . .	1 part.
Glycerine	. . .	1 part.
Water	. . .	1 part.

(iv) *To fix and preserve.**

(a) Alcohol (70%)	. . .	85 c.c.
Formaldehyde (40%)	. . .	10 c.c.
Acetic acid (glacial)	. . .	5 c.c.

(b) Formaldehyde (4%).

(v) *Tissues which have been embedded in wax* may be preserved just as they are, in the block of wax.

(vi) *To preserve green colour of museum specimens.*

(a) Dissolve cupric acetate to saturation in acetic acid (comm.). Decant. Mix with an equal vol. of distilled water (for delicate plants use 4 vols. of water). Boil in a glazed earthenware, or glass vessel. Drop specimen into the hot solution. Boil gently ($\frac{1}{2}$ hour for strong plants and 5 mins. for delicate plants). Remove with wooden forceps. Wash thoroughly in running water for 12 hours. Mount in formalin (2%).

(b)**Solution A.*

Alcohol (industrial)	. . .	90 c.c.
Formaldehyde (40%)	. . .	5 c.c.
Glycerine	. . .	2.5 c.c.

* Adapted from *Gerrard's Bulletin*, May 1939. T. Gerrard & Co.

PRESERVATION OF MATERIAL

Solution B.

Acetic acid (glacial)	.	.	2.5 c.c.
Copper chloride	.	.	10 gr.
Uranium nitrate	.	.	1.5 gr.

Mix equal volumes of solutions A and B.

(vii) *To preserve green colour of algae.* See "Algæ" (iii) (p. 312).

(viii) *To preserve fungi.* See "Fungi" (p. 317).

Polyzoa.

Alcohol (70%).

Porifera.

Alcohol (75%), or (90%).

Raia. See under "Scyllium" (below).

Rana.

(i) Formaldehyde (3%).

(ii) See "Animal Tissue" (ii) (p. 313).

(iii) *Spawn.* See "Frog Spawn" (p. 316).

Scyllium.

(i) 1° Formaldehyde (10%).

2° " (5%).

3° " (3%).

(24 hours).

(7 days).

(stock).

(ii) See "Animal Tissue" (ii) (p. 313).

Note: First open abdominal cavity and pericardial cavity and remove a $\frac{1}{4}$ " square from the roof of the cranium.

Sea Anemone. See "Anemone" (p. 313).

Sea Fir. See "Sertularia" (p. 321).

Sea Mat. See "Polyzoa" (above).

Sea Mouse. See "Aphrodite" (p. 315).

Sea Pen. See "Pennatula" (p. 318).

Sea Slug. See "Doris" (p. 316).

Sea Squirt. See "Tunicates" (p. 322).

Sea Urchin. See "Echinus" (p. 316).

Seaweeds. See "Algæ" (p. 312).

Sertularia.

Alcohol (70%).

Skins of Animals.

- (i) Remove all traces of fat, keep free from damp, and rub in a preservative frequently. Arsenical soap (Bécoeur's) (p. 229) is the best preservative, but great care must be exercised in its use, or poisoning of the operator may occur. Browne's soap (p. 232) is a non-poisonous substitute. Burnt alum and saltpetre (p. 285) are of value, but tend to make the skin hard.
- (ii) If necessary to skin the animal, make as few incisions as possible.
 - (a) *Large Mammals.* Make a full-length incision along the mid-ventral line, and incisions on the insides of the limbs which are severed at the knees (the lower parts being retained). Withdraw the hind limbs first. Keep the skull in turpentine.
 - (b) *Small Mammals.* Make one incision only, from the middle of the mid-ventral line to the pectoral region.
 - (c) *Birds.* Make an incision under the skin of a wing. Plug the mouth with cotton wool and leave the skull in.
 - (d) *Small Reptiles.* Make an incision at the point where skull is attached to cervical vertebræ. Evert the decapitated trunk through the mouth.
 - (e) *Large Reptiles.* As mammals.

(f) *Fish*. Make an incision along the more damaged side from tail to gills. *Keep the skin damp while removing myotomes*. Rub in preservative and fill cavity with sawdust.

(iii) See also "Preservation of Museum Specimens," Dollman, *School Science Review*, XVIII, 69, 91; Oct. 1936.

Slugs.

Alcohol (60%).

Sponges. See "Porifera" (p. 320).

Starfish. See "Asterias" (p. 315).

Taxidermy. See "Skins of Animals" (p. 321).

Tunicates.

Alcohol (70%).

APPENDIX

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* These publications are a constant source of hints and tips gained from practical experience. Issues Nos. 58, 59, 60, and 61 of the *School Science Review* will be found especially valuable.

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